博 士 論 文 の 内 容 の 要 旨
Abstract of Doctoral Dissertation

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論文題目 Dissertation Title	Effect of environmental condition on capsaicinoid, sugar content and expression of capsaicinoid biosynthesis genes in chili pepper (*Capsicum* spp.) （トウガラシ（*Capsicum* spp.）のカプサイシノイドおよび糖含量ならびにカプサイシノイド合成関連遺伝子の発現に及ぼす環境の影響 ）

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Chili pepper (*Capsicum* spp.) is mainly used in the food industry. *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescen* are the five domesticated species of the genus *Capsicum*, containing more than 30 species (Moscone et al., 2007). Capsaicinoid is the major chili compound used in the pharmaceutical and medical industries. Sweetness is another basic taste in chili pepper due to glucose and fructose. Chili plant growth, yield, and chemical content are changed by environmental factors such as drought stress, salinity stress (Rathnayaka et al., 2021a and 2021b), parthenocarpy (Kondo et al., 2021), and light condition and fertilizer condition (Buczkowska et al., 2013). The objectives of the present study were to determine the relationship between P fertilizer with capsaicinoid, sugar, total sugar content, and Brix percentage and determine the expression level of the capsaicinoid biosynthesis gene in excess P fertilizer application. According to the climate change models predictions, the air temperature will increase between 1-4°C by the end of the 21st century because of the greenhouse effect. High temperature is a stress for plants, and the components like capsaicinoids, sugar, and glutamic acid produced by the plant will change as a response to such stresses. Therefore, the other objective of the present study was to determine the effect of temperature stress on capsaicinoid, glucose, total sugar, Brix, and glutamic acid content.

All the experiments were carried out in the Education and Research Center of Alpine Field Science, Faculty of Agriculture, Shinshu University as three experiments. Experiment 1- The effect of soil phosphorous on capsaicinoid levels and sugar content in the fruits of three *C. annuum* varieties ('Takanotsume', 'Sapporo Oonaga Namban' and 'Shishito') and one *C. chinense* variety ('Habanero') were investigated. In 2018 and 2020, different levels of phosphorus fertilizer were applied and the capsaicinoid of the fruits was measured by using high-performance liquid chromatography (HPLC).

Glucose and total sugar were analyzed using a portable spectrophotometer (RQ flex). Brix value was measured using a portable digital refractometer. Experiment 2 - As the second part of the P application experiments, the number of seeds, placental septum weight, and capsaicinoid content in 'Takanotsume', 'Sapporo Oonaga Namban', and 'Shishito' (only used in 2020) chili pepper varieties were investigated under different phosphorus fertilizer treatments in 2020 (60, 300 and 600 $g \cdot m^{-2}$) and in 2021 (100 and 600 $g \cdot m^{-2}$). Furthermore, relative expression levels of 18 capsaicinoid biosynthesis genes were tested using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) in 2021. Experiment 3 - The effect of

temperature stress on capsaicinoid, glucose, total sugar, Brix, and glutamic acid content in 'Takanotsume' (2021 and 2022), 'Habanero', and 'Himo' (2022) varieties were investigated. Experiments were conducted using two temperature treatments under greenhouses (temperature stress) and in open field conditions (control). The capsaicinoid content, glucose, total sugar, glutamic acid contents, and Brix percentage were measured.

While investigating the effect of phosphorus fertilizer level in Experiment 1, the capsaicinoid content increased from 100 to 200 $g \cdot m^{-2}$ and then decreased at the 300 $g \cdot m^{-2}$ phosphorus fertilizer applied in 'Takanotsume', 'Sapporo Oonaga Namban' and 'Habanero'. These results reveal that the capsaicinoid content increased with the increment of phosphorus fertilizer and tends to decrease with excess phosphorus application. There was a tendency for the total sugar and glucose content to increase slightly when the plants were grown in soil with high phosphorus (300 $g \cdot m^{-2}$ and 600 $g \cdot m^{-2}$). In Experiment 2, with the increasing amount of P fertilizer added, the number of seeds and placenta dry weights did not change, but the capsaicinoid content was significantly lower in all varieties except 'Shishito'. According to the qRT-PCR of 18 capsaicinoid biosynthesis genes, the genes were divided into four groups based on their expression patterns. Group 1 showed higher gene expression of *Pun1*, *pAMT*, *ACL*, and *CaKR1* genes in plants grown in 100 g·m-2 P-treated soil for both cultivars at 20 days after flowering (DAF). The group 2 genes, *WRKY9*, *BCKDH*, *KAS I*, *CaMYB31*, *HTC*, *KAS III*, and *BCAT* showed higher expression in 'Takanotsume' under 100 $g \cdot m^{-2}$ P fertilizer treatment at 20 DAF. Group 3, comprised *ACS*, *FAT* and *COMT*, showed higher expression in at least one variety grown in 100 $g \cdot m^{-2}$ P fertilizer at 30 DAF. The increased expression of groups 1, 2, and 3 genes induces increased pungency of chili peppers grown in 100 $g \cdot m^{-2}$ P fertilizer. In Experiment 3, capsaicinoid content was significantly higher in temperature stress conditions than the control condition of all varieties in both years except 'Himo' at 20 DAF. In both years, total sugar content, Brix percentage, and glutamic acid content were significantly higher in the temperature stress condition than in the control condition. Only glucose content had an inverse effect showing that all varieties of glucose content were significantly lower in the temperature stress condition than in the control condition.

The average number of seeds did not increase or decrease for the 100 $g \cdot m^{-2}$ P fertilizer level or 600 $g \cdot m^{-2}$ P fertilizer level in Experiment 2. However, capsaicinoid content decreased at 600 $g \cdot m^{-2}$ P fertilizer level (excess P) than at 100 $g \cdot m^{-2}$ P fertilizer level. This means the capsaicinoid content was reduced, not because of the trade-off with the lignin, but because the capsaicinoid synthesis ability of the placental septum has been reduced in excess P. As a result of Experiment 3, high-temperature stress condition leads to reduce the seed number. Then, the capsaicinoid content became high because of the trade-off. Therefore, it is clear that the temperature stress does not directly affect capsaicinoid biosynthesis, but it indirectly affects to increase of the capsaicinoid. Overall, the present study revealed that the P level and temperature stress, respectively, directly and indirectly, affect the taste components, including capsaicinoid. The high-temperature stress reduces seed number and increases capsaicinoid content in chili pepper fruit. On the other hand, phosphate fertilizer application also similarly affects capsaicinoid content in the fruit but does not change seed number. Continuous addition of P fertilizer to the cultivation fields does not produce quality chili fruits. Instead, farmers could measure the available soil P to avoid adding extra P, which will also be cost-effective. At the same time, for the farmers who want to produce more pungent chili, this will prevent adding excess P to the fields, reducing the pungency. The pungency increases when considering the high temperature stress than the average environmental temperature. Based on our results, it became clear that the farmers can produce high pungent quality chili fruits if they provide temperature stress to the chili plants, but we cannot precisely conclude the optimum temperature to produce the optimum quality. Therefore, more research has to be done to investigate the optimum temperature for chili cultivation.