Elucidation of the physiologic and genetic characteristics of autonomous fruit-set under high temperature in chili pepper

（トウガラシにおける高温期の自動着果性の生理的および遺伝的特性の解明）

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Abstract

Capsicum is an important crop used as spices, vegetables, and ornamental crops. Fruit vegetables of the Solanaceae family, including *Capsicum*, set fruit without fruit set promotion treatment (autonomous fruit set). However, it is predicted that pollen and embryo sac fertility will decline, and the rate of fruit set will decrease as the air temperature rises owing to global warming (Hedhly et al., 2008; Driedonks et al., 2016; Mesihovic et al., 2016). Even chili peppers cannot maintain their ability to set fruits autonomously under high temperatures. The fruit set rate of *Capsicum* plants generally decreases under high temperature conditions of 33°C or higher (Erickson and Markhart, 2002; Garruna-Hernandez et al., 2012). Therefore, autonomous fruit set ability, even under high temperatures is required, but a search for genetic resources that can do this has not been conducted. In this study, I first found *Capsicum* genotypes that exhibited autonomous fruit set under high temperatures, and then obtained the basic knowledge of their physiologic and genetic mechanisms.

In Chapter 1, I searched for cultivars of *Capsicum* plants with high autonomous fruit sets under high temperatures. A total of 13 cultivars of *C. annuum*, *C. bacattum*, *C. frutescens* and *C. chinense* were cultivated in a greenhouse under a natural condition in summer. Consequently, the percentages of autonomous fruit set of the two cultivars of *C. annuum*, ‘Takanotsume’ and ‘Goshiki-Kyokko’, were significantly higher under high temperatures. The percentage of autonomous fruit set was highly correlated with the pollen germination rate (r = 0.63) within the *C. annuum* cultivar group tested. Although the percentage of autonomous fruit set and pollen germination rate of the two *C. chinense* cultivars ‘Sy-2’ and ‘No.3686’ were extremely low, both were high in the F_1 hybrids. Thus, a significant difference between cultivars was found in the autonomous fruit set rate under high temperatures, suggesting that there is a mechanism for genetically controlling the autonomous fruit set under high temperatures. In particular, the difference in pollen germination rate difference was likely to be related to the autonomous fruit set. Thus, it was considered important to focus on the pollen germination rate in order to clarify the autonomous fruit set under high temperatures.

In Chapter 2, focusing on the F_1 hybrids of ‘Sy-2’ × ‘No.3686’, which have high autonomous fruit set ability under high temperatures (found in Chapter 1), I investigated the percentage of fruit set and reproductive traits related to autonomous fruit set. Moreover, reciprocal crossing by artificial

pollination was performed to clarify whether male or female factors were involved in the autonomous fruit set under high temperatures. The autonomous fruit set ability under high temperatures of F_1 hybrids of 'Sy-2' and 'No.3686' was observed in different years. The parent cultivars of 'Sy-2' and 'No.3686' did not set any fruit in summer, whereas the F_1 hybrid did, although both the parent cultivars and the F_1 hybrids set fruit after early autumn when the maximum temperature dropped below 35°C. Thus, it was shown that only F_1 hybrids exhibited autonomous fruit set when the maximum temperature was 35°C or higher. The percentages of fruit set were higher when F_1 hybrid pollen was pollinated to the pistils of 'Sy-2' and F_1 hybrids than when 'Sy-2' and '3686' pollens were pollinated. However, no fruit set was observed when the F_1 hybrid pollen was pollinated to the pistil of 'No.3686'. Therefore, it was considered that the high autonomous fruit set ability in F_1 hybrids is closely related to the maintenance of pollen germination ability and pistil fertility under high temperatures.

In Chapter 3, the genetic analysis of the autonomous fruit set under high temperatures in the F_1 hybrids of 'Sy-2' × 'No.3686' was performed. The segregation in the percentage of fruit set and pollen germination rate in the F_2 population was investigated. Some F_2 individuals had higher percentages of autonomous fruit set than F_1 hybrids. The F_2 population was subjected to ddRAD-Seq analysis, single nucleotide polymorphism information was obtained, and association analysis was performed. A genetic region significantly associated with the percentage of fruit set and the pollen germination rate was detected on chromosome 6. Thus, a gene that regulates autonomous fruit set and pollen germination ability under high temperatures (*Male reproductive Heat tolerance 1*: *MH1*) appears to be found. In addition, a genetic region related to autonomous fruit set and pollen germination ability was observed on chromosome 3. Thus, another gene that regulates the traits appears to be found (*MH2*). F_2 individuals with the 'Sy-2'-type *MH1* allele and the 'No.3686'-type *MH2* allele exhibited higher rates of autonomous fruit set and pollen germination rate. F_2 individuals with the 'No.3686'-type *MH1* allele and the 'Sy-2'-type *MH2* allele tended to have a lower percentage of autonomous fruit set and pollen germination rates. In addition, F_2 individuals with heterozygous genotypes in each gene had the intermediate values of the autonomous fruit set and pollen germination rates between the two genotypes. Therefore, the autonomous fruit set under high temperatures exhibited in the F_1 hybrid of 'Sy-2' × 'No.3686' was thought to be mainly regulated by these two genes, *MH1* and *MH2*.

In Chapter 4, I analyzed the effects of *MH1* and *MH2* estimated in Chapter 3. The autonomous fruit set and pollen germination in the high-temperature period were investigated using an F_4 population. In addition, DNA markers designed in Chapter 3 which determine the genotype 'Sy-2' or 'No.3686' in *MH1* and *MH2*, were used for genotyping the F_4 individuals. The F_4 population was classified into four groups according to the genotype of the two genetic loci, and the trait values were compared between the groups. The group with the 'Sy-2'-type *MH1* allele and the 'No.3686'-type *MH2* allele had the highest percentages of autonomous fruit set under high temperatures, whereas the group with 'No.3686'-type *MH1* allele and the 'Sy-2'-type *MH2* allele had the lowest percentages. The other two groups had an intermediate value of these two groups; thus, the F_4 population showed an additive inheritance in the fruit set. However, the pollen germination rate showed a different inheritance type. The group with the 'Sy-2'-type *MH1* allele and the 'Sy-2'-type *MH2* allele and the group with the 'No.3686'-type *MH1* allele and the 'Sy-2'-type *MH2* allele had the lowest pollen germination rates. The group with the 'No.3686'-type *MH1* allele and the 'No.3686'-type *MH2* allele had intermediate values. Thus, the 'No.3686'-type *MH2* allele alone improved the pollen germination rate under high temperatures. However, the 'Sy-2'-type *MH1* allele was believed to improve the pollen germination rate only by interacting with the 'No.3686'-type *MH2* allele. In addition, the 'Sy-2'-type *MH1* allele improved the autonomous fruit set under a high-temperature period, presumably for factors other than the improved pollen germination rate. Therefore, it was suggested that the reason the F_1 hybrids that obtained by the hybridization of two cultivars acquired autonomous fruit set under high temperatures was not only due to the additive gene effect but also the interaction of two genes.

This research focused on the autonomous fruit set even under high temperatures without any fruit set promotion treatments. It was clarified that the autonomous fruit set under high temperatures could be genetically controlled; therefore, plant breeding may improve the fruit yield under high temperatures. I found that F_1 hybrids between 'Sy-2' and 'No.3686', both of which do not have autonomous fruit set under high temperatures, show a high percentage of autonomous fruit set. The autonomous fruit set ability under high temperatures in F_1 hybrids is owing to the maintenance of pollen germination ability and female fertility. The isolation and functional analysis of the *MH1* and *MH2* genes detected in this study are anticipated to play a significant role in promoting the breeding program of autonomous fruit set cultivars in fruit vegetables. 'Sy-2' is derived from the Seshal Islands (Yazawa et al., 1989), while 'No.3686' is derived from Colombia (Yamamoto, 1978). It was shown that the interaction of genes possessed by cultivars of different origins contributes to fertility improvement under high temperatures. Therefore, plant breeding for autonomous fruit set under high temperatures may progress significantly by focusing on the potential of existing genetic resources and the combinations of genes obtained by hybridization.