

**Developmental and Molecular Biological Dissection of a
Heat Tolerant Tomato Mutant *HT7***

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**Developmental and Molecular Biological Dissection of a
Heat Tolerant Tomato Mutant *HT7***

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論文概要
[Abstract of Thesis]

論文題目 [Thesis Title] * Developmental and Molecular Biological Dissection of a Heat Tolerant Tomato Mutant *HT7*

(耐暑性トマト変異体 *HT7* の発生生物学および分子生物学的解析)

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論文概要 [Abstract of Thesis]

Tomato (*Solanum lycopersicum*) is an undeniable importance of vegetable crop in term of the merits of fresh and industrial products. It is also well known as an incredible source of rich nutrient components such as vitamin C, β -carotene and lycopene that positively impact on human health (Bergougnoux 2014). However, the yield and quality of tomato are adversely affected as a consequence of various biotic and abiotic stresses. High temperature is one of the major global abiotic stresses, which causes the multifarious negative effects on plant morphology, physiology, biochemistry and molecular pathways at all vegetative and reproductive stages, causing the reduce of fruit set and bad quality (Hasanuzzaman et al. 2013, Bitra and Gerats 2013).

We isolated heat-tolerant (HT) mutants providing improved fruit production under long-term ambient high temperature among EMS tomato mutant populations, created by National BioResource Project. In this project, we focused on the *HT7* produced normal fruits with seeds in heat stress (HS) to understand the developmental and molecular dissection of HT mutant lines. Mutant and WT were evaluated in two fixed cultivated conditions, HS (35°/25 °C) or control (CO) (25 °C), 16 h/8 h light/dark, 60.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and opened greenhouse in summer 2018.

Mutant *HT7* expressed several unique traits under long-term exposing to elevated temperature: *HT7* kept the narrow plant canopy with producing fewer lateral shoots; *HT7* had around 30-40 flowers per plant; rate of fruit setting (6 %) was five times higher than WT, resulting the fruit yield of *HT7* was two times higher than WT; *HT7* consistently produced normal fruits in HS, while WT could not get fruit set for every cultivation. Typically, *HT7* fruits contained seeds

that could germinate, while 100 % of WT fruits did not have seeds. In the opened greenhouse in summer 2018, 100 % of *HT7* and WT fruits produced fruits without seeds when bud formation and anthesis were during the highest peak of summer (more than 42 °C at noon).

In HS, mutant *HT7* remained the flower structure was more suitable for self-pollination. *HT7* had less than 20 % of abnormal flowers, while WT was more than 40 %; *HT7* produced pollens within pollen sacs, which their walls opened for releasing pollens, while 40 % WT flowers did not produce pollens and pollen sac walls did not open well at anthesis. In long term HS, *HT7* produced two times higher viable pollens and total pollens than WT; *HT7* and WT pollens germinated very low in the pollen germination medium, at 7 % and 6 %, while these rates were more than 80 % in CO; *HT7* and WT pollens did not elongate pollen tubes well. In short term HS, *HT7* produced two times higher in viable pollens than WT in -1, -3, -5 days before anthesis, however, the pollen germinate rate was not significant differences. In the greenhouse, *HT7* and WT flowers had more than 85 % of pollens died due to hot temperature.

To adapt with the elevated temperature, tomatoes have established various heat responded reactions. The 2-weeks-old seedlings of *HT7* and WT died after 12 h constantly treated at 42 °C; the stomata diameters of *HT7* were the biggest in HS and smallest in GRH, while those in WT were the biggest in CO and smallest in GRH. The stomata density on leaves of *HT7* was smaller than in WT in three conditions; *HT7* released lower at 10 days and higher at 60 days in HS in total ionic leakage than WT; Fruits of *HT7* and WT released similar amount of hydrogen peroxide. The amount of Fructose, Glucose and Sucrose in *HT7* fruits were lower than in WT.

Transcription factors, heat shock proteins and other related to heat tolerance play important roles in thermotolerance. In leaves, *HT7* expressed *SIHsfA1a*, *SIHsfA1d* genes lower than WT, in contrasted, *HT7* expressed *SIHsfA1b1* and *SIHsfA1b3* higher than WT; The relative gene expression levels of *HT7* in *SIHsp21* and *SIHsp01* were three to four times higher than WT. However, in the anther cone, the expression of *SIHsfA1a*, *SIHsfA1b1* and *SIHsp101* were not significant differences between *HT7* and WT. In the greenhouse, the expression levels of ABCDE model genes were different, *HT7* expressed lower in *SIAGL6* and *SINAM2* genes, while higher in *SILePI*, *SIMC* and *SITM6* than in WT. Regarding to leaf senesce genes, *RbcL*, *SENU3* and *DREB2A* expressed highest at 3 hours in HS. *HT7* expressed *RbcL* and *DREB2A* lower than WT at 7 days in HS.

Detecting the heat tolerant responsible gene(s) in mutant *HT7* is one important aim. The whole genome sequencing by NGS was carried out on the bulked DNA extracting from the leaves of F₂ population based on the fruit setting number. Total reads 327,921,773 (WT – like F₂), 326,421,481 (*HT7* – like F₂), 151,100,978 (WT), 158,203,158 (*HT7*) were called. Sanger sequencing and restriction enzyme digestion were performed in different mutative positions, however, the strongly responsible gene(s) were not detected yet.

In conclusion, mutant *HT7* had more normal flowers compatibly for self-pollination, narrow plant canopy, higher viable pollens, higher fruit setting rate, higher fruit number per plant resulting higher fruit yield. Particularly, *HT7* produced normal fruits containing seeds and stayed green in HS. Therefore, *HT7* can be a prominent breeding material for enhancing heat tolerance.