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INDICATOR CANDIDATE TRAITS FOR AUTONOMOUS FRUIT SET ABILITY UNDER HIGH TEMPERATURES IN *CAPSICUM*

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ABSTRACT

"Autonomous fruit set" refers to self-pollination and fruit set without pollen vectors such as vibration or insects. Autonomous fruit set under high-temperature stress is an important breeding goal as climate change can reduce fruit yields in *Capsicum*. We screened *Capsicum* cultivars for autonomous fruit set ability in a greenhouse environment and investigated pollen germination, viability, pollen grains number, chlorophyll fluorescence (Fv/Fm), style length, anther cone length, and anthesis stage under high temperatures in order to identify indicator traits for screening more genotypes with autonomous fruit set ability. The fruit set of the 'Takanotsume' (57.7 ± 20.6%) and 'Goshiki Kyokko' (52.2 ± 14.2%) cultivars (both *C. annuum*) were higher than those of other cultivars. Correlation analysis showed that pollen germination had the highest correlation with fruit set in *C. annuum* cultivars (r = 0.63). These results indicate that 'Takanotsume' and 'Goshiki Kyokko' are useful cultivars for novel breeding programs focusing on autonomous fruit sets under high temperatures, and pollen germination in *C. annuum* was a convincing candidate for an indicator trait of autonomous fruit set ability under high temperatures.

Key words: chili pepper, fruit set, greenhouse cultivation, heat stress, ornamental pepper, pollen germination, sweet pepper

INTRODUCTION

Global climate change is causing a gradual increase in ambient temperatures. Climate models predict that by the end of the 21st century, average temperatures will continue to rise by 0.3–1.7 °C or even by 2.6–4.8 °C as estimated by Representative Concentration Pathways scenarios 2.6 and 8.5 (IPCC 2014), respectively. Tubiello et al. (2007) estimated that the gradual rise in temperature predicted for the first half of the 21st century could lead to an increase in crop yield in high-temperate and high-latitude regions and a decrease in yield in semiarid and tropical regions. The additional warming expected for the second half of the 21st century would adversely affect yields globally. In fact, the negative effects of climate change on the yields of some crop species in some areas have already been reported (Lobell & Asner 2003; Peng et al. 2004). Reproductive ability in plants is expected to decrease as temperature increases because of global warming (Hedhly et al. 2008; Driedonks et al. 2016; Mesihovic et al. 2016), resulting in decreased yields.

The *Capsicum* genus includes important crops such as chili, bell pepper, and ornamental forms. This genus belongs to the Solanaceae family and consists of several wild species and five domesticated species: *C. annuum*, *C. chinense*,

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C. frutescens, *C. pubescens*, and *C. baccatum* (Bosland & Votava 2000). *C. annuum* is the most widely cultivated species in the world and includes both pungent and nonpungent cultivars. Most cultivars of *C. chinense* and *C. frutescens* are highly aromatic and pungent (Bosland & Votava 2000) and are often produced in tropical and subtropical regions. Recently, these *Capsicum* species have also been cultivated in temperate regions such as Japan, occasionally in greenhouses.

Self-pollination that occurs when pollen falls to the stigma in the absence of a pollen vector is called "autonomous self-pollination", "autonomous autogamy" (Richards 1986), or "autofertility" (Yashiro et al. 1999). In greenhouse cultivation, there are no pollinators or wind unless artificially introduced. "Autonomous fruit set" refers to self-pollination and fruit set without any promotive treatments. Tomatoes and chili peppers have the ability to set fruits without stimulation also in greenhouses. In particular, we considered that chili peppers have a strong ability to autonomous fruit set since they are actually cultivated without artificial pollination. However, under high temperatures, even chili peppers cannot maintain their ability to set fruits autonomously in greenhouses.

The fruit set and reproductive abilities decrease under high temperatures, which is a major problem for agricultural productivity in Solanaceae species, including *Capsicum* spp. The fruit set and pollen viability of *C. annuum* were shown to decrease under a mild heat treatment at 33 °C for 120 h (Erickson & Markhart 2002), whereas in *C. chinense*, such a decrease was observed at temperatures above 35 °C (Garruña-Hernández et al. 2012). There are numerous traits related to fruit set under high temperatures. For example, pollen germination and pollen viability, as well as flower morphology – both style, anther length, anther dehiscence, pollen dispersal, and ovule fertility – affect fruit set under high-temperatures conditions (Levy et al. 1978; Sato et al. 2006). Attempts have been made to search for high-temperature-tolerant genotypes having genes associated with this trait (Reddy & Kakani 2007; Gajanayake et al. 2011; Ayenan et al. 2021) or related genes (Xu et al. 2017; Sun et al. 2019) and to breed heat-tolerant cultivars by introgression of genes (Rani et al. 2021; Usman et al. 2018) in Solanaceae.

Previously, we found an F_1 hybrid of *C. chinense* with autonomous fruit set ability in high-temperature greenhouses, although both parent cultivars 'Sy-2' and 'No. 3686', which seldom set fruit autonomously under high temperatures (Yamazaki & Hosokawa 2019). However, little is still known about the genotype of *Capsicum* with the ability of autonomous fruit set in hot climate and in high-temperature greenhouses. Consequently, more *Capsicum* genotypes with autonomous fruit set ability in high greenhouse temperatures should be further explored. Thus, the first aim of this study is to identify cultivars that can set fruit autonomously under high-temperature stress. In addition, indicator traits are needed to screen more genotypes that have autonomous fruit set ability under high temperatures. According to Zhou et al. (2015), the chlorophyll fluorescence parameter Fv/Fm can be used to select tomato genotypes for their thermotolerance in autonomous fertility. The second aim is to identify indicator traits in order to screen and find more genotypes with autonomous fruit set abilities under high temperatures. Therefore, we investigated various traits related to the fruit sets and tested the correlation between each trait, and autonomous fertility.

MATERIALS AND METHODS

Plant materials and growth conditions in the greenhouse and in the incubator

Thirteen *Capsicum* cultivars of the pungent, nonpungent, and ornamental peppers commonly cultivated in Japan were used for screening their ability to autonomous fruit set under high temperatures (Table 1). The genotypes for the experiment were selected on the basis of their importance in Japanese horticulture and the results of the previous experiment (unpublished data). We admittedly stated in previous experiments that 'Peruvian Purple' seldom sets fruits under high temperatures, but we would like to reconfirm this information because it is attractive for cultivation due to its compact growth. The edible (pungent and nonpungent) cultivars of *C. annuum* were represented by 8 or more individuals, the ornamental cultivars of *C. annuum* by 4–5 individuals, and the species other than *C. annuum* by 3–5 individuals. The seedlings were planted in the greenhouse at the Kizu Experimental Farm of Kyoto University (34°73′N, 135°84′E). The greenhouse was covered with an insect-proof net to prevent pollination by insects. No external treatments to promote fruit sets, such as vibration and hormonal treatments, were performed. Temperature was recorded every 10 minutes (Fig. 1A, B) using a temperature sensor (SHA-3151; T&D Corporation, Nagano, Japan) connected to a data logger (TR-72wb-S; T&D Corporation). The survey periods under high temperatures were from July 8 to 28, and those under moderate temperatures were from September 16 to October 7 in 2021.

Separate greenhouse and incubator experiments were performed to compare fruit set and pollen germination on ‘Takanotsume’ and ‘Peruvian Purple’ (greenhouse) and ‘Takanotsume’ and ‘Murasaki’ (incubator). We would like to reconfirm a high percentage of fruit setting of ‘Takanotsume’ under high temperatures (Table 2). ‘Peruvian Purple’ and ‘Murasaki’ were chosen due to their genetically close relation and because ‘Murasaki’ is attractive for their compact growth. Three months old seedlings were planted, and fruit set and pollen germination were investigated after one month of growth. An incubator (LH-240SP; Nippon Medical & Chemical Instruments, Osaka, Japan) with the high-temperature condition according to Yamazaki and Hosokawa (2020) was used. The light and temperature regimes were 7:00–10:00, 30 °C and light, 10:00–16:00, 35 °C and light, 16:00–19:00, 30 °C and light, and 19:00–07:00 20 °C and dark.

Table 1. *Capsicum* cultivars used for screening of autonomous fruit set ability under high temperatures in the greenhouse

Cultivar	Species	Number of individuals	Cultivar type
‘Takanotsume’	*C. annuum*	8	pungent
‘Nikko’	*C. annuum*	9	pungent
‘Furu-pi-yellow’	*C. annuum*	11	nonpungent
‘Furu-pi-red’	*C. annuum*	11	nonpungent
‘Onikis Red’	*C. annuum*	5	ornamental
‘Colorful Mix’	*C. annuum*	4	ornamental
‘Uchu Cream Red’	*C. annuum*	5	ornamental
‘Goshiki Kyokko’	*C. annuum*	5	ornamental
‘Shima Togarashi’	*C. frutescens*	4	pungent
‘Bishop’s Crown’	*C. baccatum*	5	ornamental
‘Sy-2’	*C. chinense*	3	pungent
‘No. 3686’	*C. chinense*	3	pungent
F_1 hybrid of ‘Sy-2’ × ‘No. 3686’	*C. chinense*	3	pungent

Table 2. Reproductive and vegetative trait parameters under high and moderate temperatures under cultivation in the greenhouse

Cultivar	Pollen number (×10³)			Pollen viability (%)	Pollen germination (%)			Fruit set (%)		
Temperature	High	Moderate	H/M	High	High	Moderate	H/M	High	Moderate	H/M
'Takanotsume'	28.9±11.3 b	19.5±3.1 ac	1.48	7.7±7.9 a	6.5±4.2 ab	7.1±3.3 a	0.91	57.7±20.6 a	32.2±15.7 a	1.79
'Nikko'	29.9±8.0 b	13.8±6.6 bcd	2.17	2.8±2.8 a	1.6±1.1 bc	1.4±0.7 a	1.14	18.2±10.3 bc	19.7±14.4 a	0.92
'Furu-pi-yellow'	55.5±15.7 a	41.4±24.4 a	1.34	0.5±0.7 a	1.7±2.0 bc	2.9±2.0 a	0.61	25.5±17.7 b	36.3±15.8 a	0.70
'Furu-pi-red'	51.5±13.8 a	38.2±11.5 acd	1.35	7.4±14.2 a	0.6±0.9 c	1.0±0.8 a	0.60	7.7±8.5 c	28.3±9.0 a	0.27
'Onikis Red'	26.2±5.3 b	-	-	9.6±7.1 a	1.4±1.3 bc	0.5±0.6 a	2.74	9.2±9.5 bc	18.7±14.0 a	0.49
'Colorful Mix'	35.8±13.5 ab	-	-	8.6±7.7 a	0.4±0.4 bc	0.8±1.1 a	0.57	7.3±4.7 bc	15.4±10.4 a	0.47
'Uchu Cream Red'	23.0±16.7 b	-	-	14.9±10.6 a	0.4±0.3 bc	1.6±0.8 a	0.25	4.4±6.7 bc	3.5±3.8 a	1.25
'Goshiki Kyokko'	23.0±9.5 b	-	-	6.3±8.6 a	6.3±4.7 ac	3.2±1.7 a	1.97	52.2±14.2 a	33.3±12.8 a	1.57
'Shima Togarashi'	21.5±12.6 b	-	-	11.7±7.0 a	12.5±8.0 a	6.9±5.0 a	1.83	1.5±2.9 bc	16.5±13.0 a	0.09
'Bishop's Crown'	38.4±8.6 ab	45.6±15.0 ab	0.84	2.2±2.6 a	3.8±3.1 bc	16.1±7.0 a	0.24	0.0±0.0 c	20.9±6.8 a	0.00
'Sy-2'	20.7±9.5 b	9.0±2.7 def	2.30	5.2±1.5 a	3.3±1.6 bc	6.2±3.1 a	0.53	0.0±0.0 bc	-	-
'No.3686'	10.0±5.3 b	5.0±2.0 f	2.00	6.4±7.2 a	3.5±4.8 bc	6.4±2.8 a	0.55	0.0±0.0 bc	-	-
F_1 hybrid	10.7±4.0 b	4.7±2.7 ef	2.28	15.0±3.4 a	6.1±3.0 ac	5.9±2.9 a	1.02	7.9±8.0 bc	31.2±6.4 a	0.25

Cultivar	Anther length (mm)			Style length (mm)			Anthesis stage			Fv/Fm		
Temperature	High	Moderate	H/M	High	Moderate	H/M	High	Moderate	H/M	High	Moderate	H/M
'Takanotsume'	2.4±0.2 bc[z]	2.8±0.2 ac	0.87	6.6±0.4 a	6.5±0.6 ab	1.01	4.0±0.0 a	3.9±0.4 a	1.03	0.64±0.14 d	0.78±0.02 a	0.82
'Nikko'	2.5±0.1 b	2.5±0.2 bcd	1.00	7.2±0.4 a	7.3±0.3 a	0.99	4.0±0.0 a	4.0±0.0 a	1.00	0.76±0.04 ab	0.79±0.01 a	0.96
'Furu-pi-yellow'	2.9±0.3 a	2.6±0.3 a	1.12	6.6±0.6 a	5.7±0.4 cde	1.17	3.8±0.7 a	4.0±0.0 a	0.95	0.78±0.01 a	0.77±0.03 a	1.01
'Furu-pi-red'	2.9±0.3 a	3.1±0.2 acd	0.95	5.7±0.4 b	4.7±0.9 bc	1.21	4.0±0.0 a	3.7±0.7 a	1.08	0.77±0.01 a	0.78±0.01 a	0.99
'Onikis Red'	1.5±0.1 de	1.9±0.5 ef	0.80	4.2±0.6 d	4.3±0.4 ef	0.97	4.0±0.0 a	4.0±0.0 a	1.00	0.65±0.02 bd	0.72±0.09 a	0.90
'Colorful Mix'	2.0±0.2 cd	2.2±0.4 def	0.92	4.1±0.7 de	4.6±0.3 df	0.90	4.0±0.0 a	4.0±0.0 a	1.00	0.61±0.02 d	0.78±0.03 a	0.78
'Uchu Cream Red'	2.4±0.4 bc	2.3±0.2 ce	1.03	4.1±0.6 de	5.4±0.4 cd	0.77	4.0±0.0 a	4.0±0.0 a	1.00	0.63±0.11 cd	0.55±0.17 b	1.15
'Goshiki Kyokko'	2.1±0.2 c	2.1±0.1 def	1.01	5.6±0.2 bc	5.2±0.6 cde	1.06	4.0±0.0 a	4.0±0.0 a	1.00	0.72±0.03 ad	0.77±0.03 a	0.94
'Shima Togarashi'	2.4±0.3 bc	2.3±0.2 ce	1.03	5.3±0.2 bc	5.4±0.4 cde	0.99	2.3±1.0 b	4.0±0.0 a	0.56	0.72±0.05 ad	0.78±0.02 a	0.92
'Bishop's Crown'	3.0±0.1 a	3.0±0.1 ab	0.99	4.7±0.4 cd	4.5±0.3 df	1.05	2.3±1.2 b	3.9±0.2 a	0.58	0.77±0.02 ab	0.79±0.01 a	0.97
'Sy-2'	2.1±0.3 bc	2.0±0.3 def	1.07	3.0±0.2 ef	3.5±0.3 f	0.84	2.2±0.7 b	3.7±0.3 a	0.60	0.77±0.03 abc	0.80±0.01 a	0.97
'No.3686'	1.5±0.1 e	1.5±0.2 f	0.96	1.3±0.3 g	1.4±0.2 g	0.91	2.1±0.1 b	3.4±0.7 a	0.61	0.79±0.00 ab	0.80±0.01 a	0.99
F_1 hybrid	2.0±0.1 ce	1.8±0.5 ef	1.08	2.6±0.1 f	2.0±0.5 g	1.27	4.0±0.0 a	4.0±0.0 a	1.00	0.80±0.01 ab	0.81±0.01 a	0.98

[z]Values are the mean ± SE of each cultivar. Different letters indicate significant differences at the 5% level by ANOVA and Tukey−Kramer test. The sample size of each cultivar was shown in Table 1. Lack of data for 'Sy-2' and 'No. 3686' concerning the fruit set resulted from the lack of fruiting caused by too low temperature for *C. chinense* at the end of the moderate season. H/M mean represents the ratio of the values under the high temperature to the moderate temperature

Measurement of the autonomous fruit set percentages

At the beginning of the experiment, five young branches without any fruits were marked. On the last day, fruits of these branches were harvested and the number of fruits and traces of falling flowers were counted. The percentage of fruit set was calculated based on these data.

Evaluation of number, viability, and germination percentages of pollen

On the day of the anthesis (when the petals open), two flowers from each individual were collected between 9:00 and 11:00. Pollen viability was evaluated through the FDA/PI staining method described in Ascari et al. (2020). Pollen grain numbers were counted using a Thoma hemocytometer (Erma, Saitama, Japan). Pollen germination percentages were calculated based on the method reported by Yamazaki and Hosokawa (2019). Pollen was suspended in the liquid medium, which included 5% sucrose and 100 ppm boric acid, and agar medium was prepared by adding 1% agar to it. The liquid medium with suspended pollen was spread on the agar medium and left overnight in the incubator. The temperature for greenhouse cultivation was set at 25 °C. The incubation temperatures for incubator cultivation were 25 °C, 30 °C, 35 °C, and 40 °C.

Evaluation of flowers' morphology

Style length and anther length were measured at the same time using an electronic caliper. On the day of flowering, the anther stages, ranging from 1 to 4, were determined as described in Yamazaki and Hosokawa (2018). The anthers of stage 1 do not dehiscence at all. The anthers of stages 2, 3, and 4 were dehisced only at the tip, half, or whole, respectively.

Measurement of Fv/Fm

Fv/Fm was measured from 14:00 to 16:00 using a handheld chlorophyll fluorometer (FluorPen FP100; Environmental Measurement Japan, Fukuoka, Japan). The night before conducting the measurement, leaves were covered with aluminum foil.

Observation of pollen tube elongation in pistils pollinated by hand

Pollen tube elongation was observed by aniline blue staining according to Yamazaki and Hosokawa (2019). The stigmas of 'Takanotsume' and 'Murasaki' cultivated in the high-temperature incubator were pollinated by their own pollen. Before the flower opening, all anthers of each flower were removed and pollinated by hand. Flowers were collected 24 h after pollination and fixed in acetic acid/ethanol (1 : 3) for 1 h. The flower samples were washed with distilled water, immersed in 8N NaOH, and kept at room temperature overnight. Pistils were stained with a 0.1% (w/v) aniline blue solution at 4 °C in the dark. The samples were placed on a glass slide and covered with a glass coverslip, followed by observation with a fluorescence microscope (BX51; OLYMPUS, Tokyo, Japan). The excitation and emission wavelengths for pollen tube observation were 330–385 nm and 420 nm, respectively, using an ultraviolet excitation filter.

Statistical analyses

Correlation analysis, t-test, χ-square test, analysis of variance method, and the Tukey–Kramer test were performed in R ver. 4.0.3 (R Core Team 2020).

RESULTS

Effect of high and moderate temperatures on the ability of autonomous fruit set

'Takanotsume' and 'Goshiki Kyokko' had the highest fruit set, more than 50%, compared to other cultivars (0–25.5%) during the high-temperature period (Table 2). There were no significant differences between cultivars grown in the moderate-temperature conditions, although both the aforementioned cultivars had the highest fruit set. The genotypes 'Sy-2' and 'No. 3686' did not set fruits, because the air temperature was too low for *C. chinense* at the end of the moderate season. Fruits of all cultivars contained many seeds, and there were no parthenocarpic fruits in both periods of high and moderate temperatures.

There were frequent days and many hours in which the temperature exceeded 35 °C during the high-temperature period (Fig. 1A), whereas the air temperature rarely exceeded 35 °C during the moderate-temperature period (Fig. 1B).

Number of pollen grains, viability, and germination

'Furu-pi-yellow' and 'Furu-pi-red' had significantly higher numbers of pollen grains than other cultivars.

There were no significant differences in pollen viability between all cultivars. Pollen germination of ‘Shima Togarashi’ was the highest among all cultivars grown under high temperatures, and ‘Bishop’s Crown’ among cultivars grown under moderate temperatures (Table 2). ‘Takanotsume’, ‘Goshiki Kyokko’, and F_1 hybrid tended to have a higher pollen germination rate than other cultivars. There were no significant differences in pollen germination under moderate temperatures.

Fv/Fm

In the plants ‘Furu-pi-yellow’ and ‘Furu-pi-red’ grown under a high-temperature period, the highest Fv/Fm value was recorded in comparison with the Fv/Fm of plants grown at the moderate-temperature period (Table 2). In ‘Takanotsume’, ‘Onikis Red’, and ‘Colorful Mix’ the value of Fv/Fm was lower under high temperatures.

Flowers development

‘Furu-pi-yellow’, ‘Furu-pi-red’, and ‘Bishop’s Crown’ followed by ‘Nikko’ had the longest anthers of any cultivar grown under high temperatures (Table 2). ‘Takanotsume’, ‘Nikko’, ‘Furu-pi-yellow’, and ‘Furu-pi-red’ had the longest style lengths. *C. chinense* cultivars had the shortest anther and style lengths. A similar trend was observed under high and moderate temperatures. The anthers of *C. annuum* cultivars matured earlier than in other species except for the F_1 hybrid under high temperatures. There was no significant difference in the anthers stages between any cultivars under moderate temperatures.

Correlation analysis for each trait under high temperatures

Correlation analysis was performed only for *C. annuum* to extract the indicator traits of the cultivars with the autonomous fruit set ability under high temperatures. A positive correlation between pollen germination and fruit set was significant in *C. annuum* cultivars (Fig. 2A). Positive correlations between anther cone length and pollen numbers, and between style length and anther cone length were also significant under high temperatures. However, any correlations between analyzed traits were not significant under moderate temperatures (Fig. 2B).

Evaluation of autonomous fruit set ability under stable high-temperature conditions in the incubator

‘Takanotsume’ had a significantly higher ($p = 0.05$) percentage of fruit set under high stable temperatures than ‘Peruvian Purple’ (Table 3, Fig. 3). ‘Takanotsume’ also had a higher pollen germination rate than ‘Peruvian Purple’. There were frequent days when the maximum temperature exceeded 35 °C during the reconfirmation survey (Fig. 1C). In addition, ‘Takanotsume’ tended to have a higher fruit set and had significantly higher pollen germination than ‘Murasaki’ in the high-temperature incubator. The pollen germination of ‘Takanotsume’ was higher than that of ‘Murasaki’ at any of the culture temperatures of 25 °C, 30 °C, 35 °C, and 40 °C (Table 3, Fig. 4A). At this time, more pollen tubes of ‘Takanotsume’ were observed in both pistils (Fig. 4B, C, D, E).

Table 3. Percentage of fruit set and pollen germination rate in the confirmation survey on plants grown in a stable high temperature in the incubator

Cultivation place	Cultivar	Fruit set (%)	Pollen germination (%)
Greenhouse	‘Takanotsume’	22.9 ± 6.0^{z}	17.1 ± 9.7
	‘Peruvian Purple’	0.0 ± 0.0	1.8 ± 0.7
	p-value	0.063^{y}	0.25^{y}
Incubator	‘Takanotsume’	16,7	17,3
	‘Murasaki’	2,8	4,0
	p-value	0.15^{x}	$<0.001^{y}$

^{z} values are the means ± SE of each cultivar;
^{y} p-value was calculated by Welch’s t-test;
^{x} p-value was calculated by χ-square test

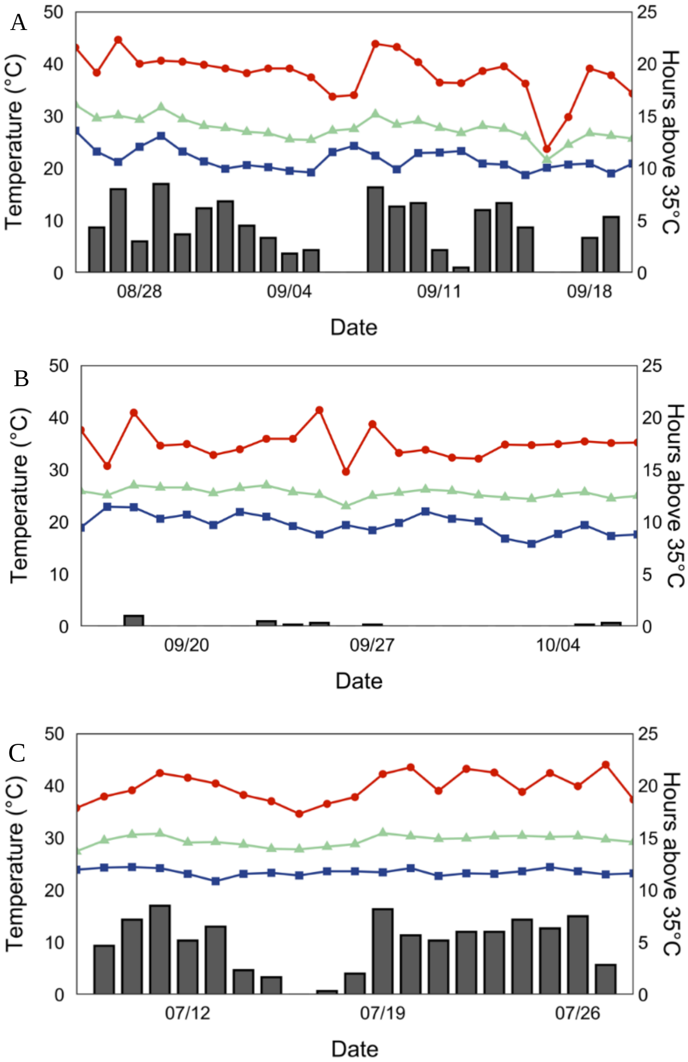

Figure 1. Air temperatures in the greenhouse during the high-temperature period for screening (A) and the moderate-temperature period (B). Air temperature during confirmation survey in the incubator (C). The line graph and the left Y-axis represent air temperature in the greenhouse. Red, green, and blue lines and circle, triangle, and square points mean highest, average, and lowest temperature on the day, respectively. Bars represent number of hours with temperature above 35 °C

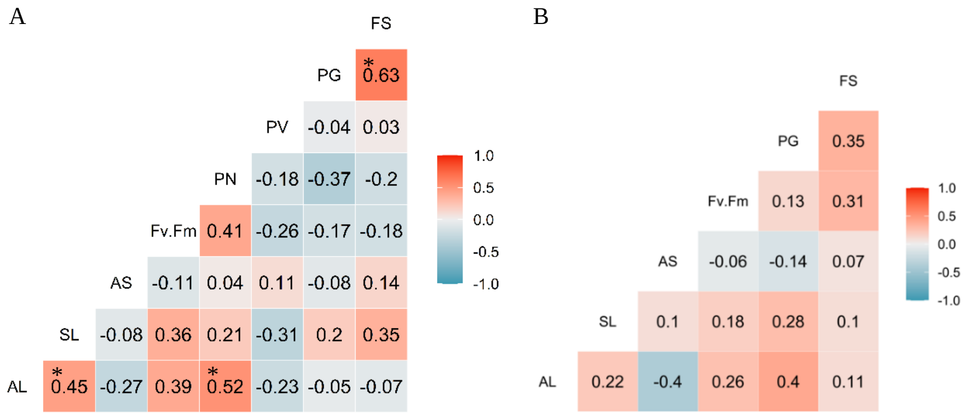

Figure 2. Correlation analysis between traits of *C. annuum* cultivars grown in the greenhouse under high-temperature conditions (A) and moderate-temperature conditions (B). The numbers in the figure show the correlation coefficient (r value) between two traits. Red and blue colors indicate positive and negative correlations, respectively. The deeper the color, the larger the absolute value of the correlation coefficient. The asterisks indicate significant correlations ($p < 0.05$). Trait observed: FS – fruit set; PG – pollen germination; PV – pollen viability; PN – pollen number; AL – anther length; SL – style length; AS – anthesis stage

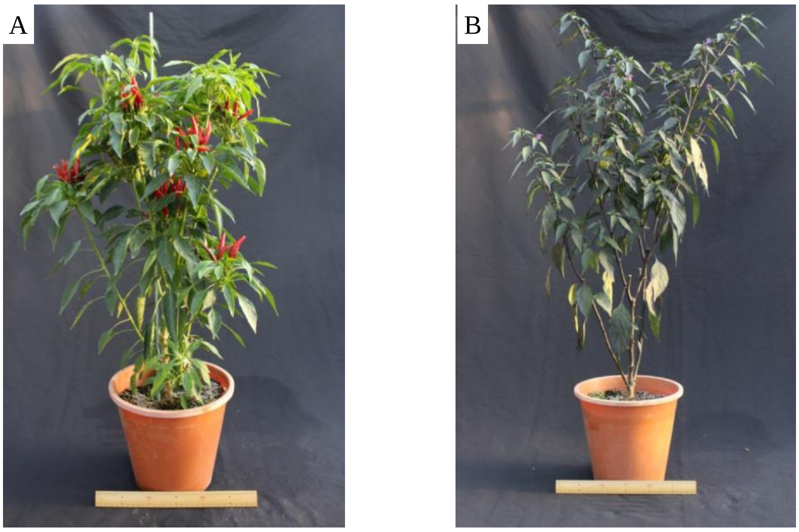

Figure 3. Difference in fruit set under high-temperature conditions of plants grown at the high-temperature period: (A) 'Takanotsume' and (B) 'Peruvian Purple'. The photos were taken on August 30, 2020. The brown ruler shows 30 cm

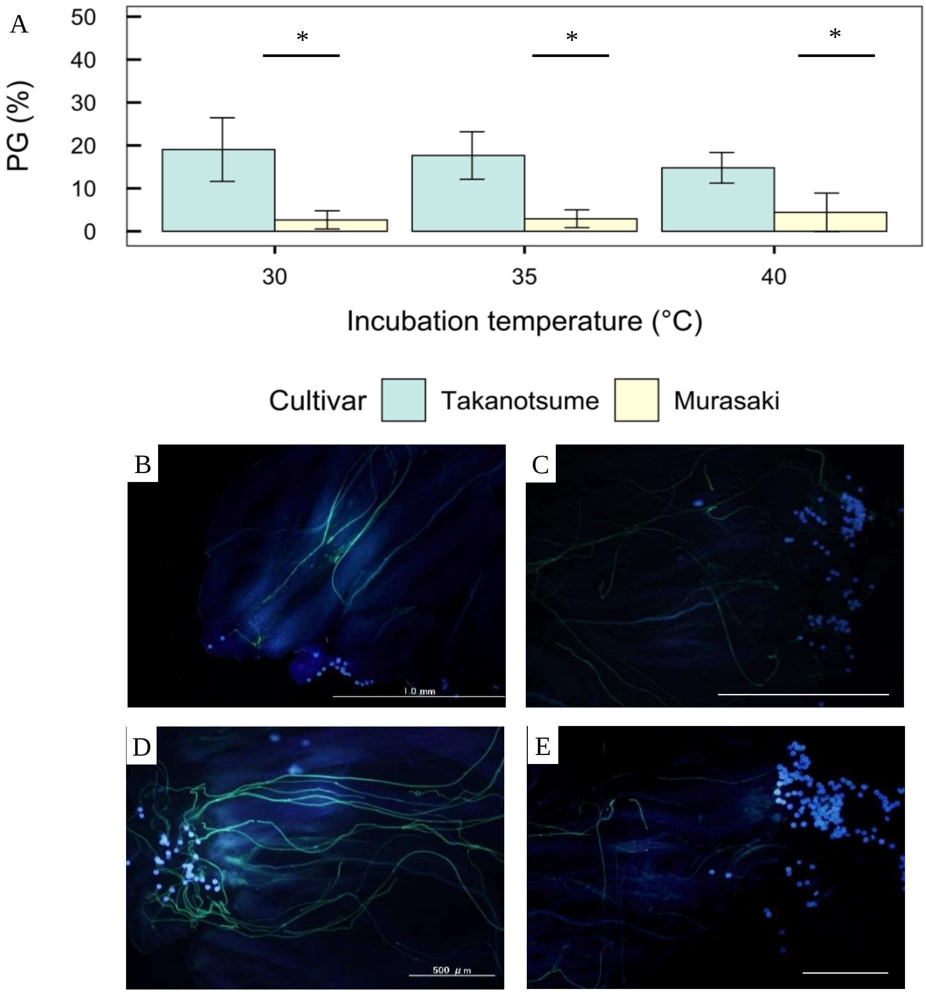

Figure 4. Germination of pollen of plants grown in the high-temperature incubator (A). Incubation temperatures were 25 °C, 30 °C, 35 °C, and 40 °C. The asterisks indicate 5% level of significance obtained with t-test. (B), (C), (D), and (E) show pollen tube elongations in the pistils of 'Takanotsume' pollinated by 'Takanotsume' (B) and 'Takanotsume' pollinated by 'Murasaki' (C). Pollen tube elongations in the pistils of 'Murasaki' pollinated by 'Takanotsume' (D) and 'Murasaki' pollinated by 'Murasaki' (E). Scale bars are 1 mm in (B) and (C), and 500 μm in (D) and (E)

DISCUSSION

The ability of autonomous fruit set decreases under high temperatures in Solanaceae species, including *Capsicum*. Most *Capsicum* species are sensitive to temperatures above 35 °C and reduce fruit set under such conditions (Erickson & Markhart 2002; Garruña-Hernández et al. 2012). Our previous study also suggested that *Capsicum* plants have low fruit sets when the maximum temperature is 35 °C or higher (Yamazaki & Hosokawa 2019). Thus, cultivars setting fruits autonomously in these conditions are required to ensure fruit vegetable yields, especially in cultivation under covers and in the context of global warming. In this study, the 'Takanotsume' grown for spices and 'Goshiki Kyokko' of ornamental value were found to potentially have a high fruit set and pollen germination ability during growing in high temperature (Table 2). The fruit set abilities of these cultivars were consistent with our past observations (Yamazaki & Hosokawa 2019). In particular, the ability of autonomous fruit set under high stable temperatures in 'Takanotsume' was reconfirmed (Table 3). In addition, 'Takanotsume' was confirmed to have a high pollen germination rate even under high temperatures (Figure 4). We previously found that the F_1 hybrid of 'Sy-2' and 'No. 3686' also set fruits autonomously under high temperatures. The autonomous fruit set ability under high temperatures of 'Takanotsume' and 'Goshiki Kyokko' were much higher than that of the F_1 hybrid.

'Takanotsume' is phylogenetically classified in the Chili group, which is closely related to the 'Hontaka' and 'Yatsubusa' cultivars (Erwin 1929). In addition, this cultivar has been classified as similar to 'Shishi Togarashi', which is closely related to 'Yatsubusa' (Konisho et al. 2005), using EST-SSR markers (Shirasawa et al. 2013). Conversely, 'Goshiki Kyokko' is classified into the Goshiki group in the Japanese cultivar classification or into the Celestial group based on Erwin's classification and is genetically different from 'Takanotsume' (Kumazawa et al. 1954). In this study, both 'Takanotsume' and 'Goshiki Kyokko' showed a similarly high fruit set and pollen germination compared to other cultivars during the high-temperature period despite their different origins. Obtaining cultivars capable of setting fruit autonomously under high temperatures in different phylogenetic groups is useful because it enhances the possibility of breeding high-temperature-tolerant cultivars. Most cultivars other than *C. annuum* had low autonomous fruit set ability under high temperatures in this study. Thus, more genotypes capable of autonomous fruit set under high temperatures should be found for breeding a wide variety of *Capsicum* cultivars.

The most reliable plant research results can be obtained under cultivation conditions. However, conducting such experiments is laborious and costly. Therefore, it is especially valuable to find marker traits related to the trait sought by the breeder. The Fv/Fm parameter related to type II photosynthesis is used, inter ales in tomatoes, as an indicator of photoinhibition (Zhou et al. 2015). However, the Fv/Fm of 'Takanotsume' was 0.64, which was lower than in some other cultivars without autonomous fruit set ability under high temperatures, although Fv/Fm varies between 0.7 and 0.8 when there is no abiotic stress in *Capsicum* (Gisbert-Mullor et al. 2021). A correlation between fruit set and Fv/Fm was not significant in the *Capsicum* cultivars in the present study (Fig. 2), suggesting that Fv/Fm cannot be used as an indicator trait correlated with autonomous fruit set ability under high temperatures in *Capsicum*.

The highest positive correlation was found between pollen germination and fruit set in some *C. annuum* cultivars. It has been previously reported that pollen germination is important for the fruit set rate at high temperatures in *Capsicum* (Aloni et al. 2001) and other fruit vegetables like tomato (Sato et al. 2000). According to Hirose (1957), the pollen germination of 'Takanotsume' was 30.4% in an open field, and in the present study, in a greenhouse during the high-temperature period, it was 6.5 ± 4.2% (Table 2). The point is that 'Takanotsume' had lower pollen germination under a high temperature in the greenhouse and in the incubator than in a field, but this parameter was still higher than that of other cultivars. Both the two cultivars, 'Takanotsume' and 'Goshiki Kyokko', which had the ability of autonomous fruit set had a high percentage of pollen germination under high temperatures. Thus, pollen germination was considered to be an indicator trait for screening genotypes with autonomous fruit set ability under high temperatures.

The pollen germination in 'Shima Togarashi' was very high, but the fruit set was low, which was possibly due to a difficult dehisce under high temperatures. Anthers dehiscence was easy in all *C. annuum* cultivars but was not in other species. Therefore, the fact that anther dehiscence does not occur under high temperatures may cause poor fruit set in *Capsicum* spp. other than *C. annuum*. It is reasonable that a decrease in the anthesis stage reduces the autonomous fruit set. Thus, the anthesis stage was also considered to be an important trait for autonomous fruit set ability under high temperatures in *Capsicum* species other than *C. annuum*.

CONCLUSIONS

'Takanotsume' and 'Goshiki Kyokko' had the ability to autonomous fruit sets under high temperatures. Thus, they are useful cultivars for breeding programs focusing on autonomous fruit set under high-temperature conditions. A positive correlation between fruit set and pollen germination was significant among some cultivars of *C. annuum*. In addition, the correlation between fruit set and anthesis stage was positively correlated among all species. Thus, pollen germination in *C. annuum* is a convincing candidate and an indicator trait for the selection of individuals able to autonomous fruit set under high temperatures.

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REFERENCES

Aloni B., Peet M., Pharr M., Karni L. 2001. The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (*Capsicum annuum*) pollen in relation to its germination. Physiologia Plantarum 112(4): 505–512. DOI: 10.1034/j.1399-3054.2001.1120407.x.

Ascari L., Novara C., Dusio V., Oddi L., Siniscalco C. 2020. Quantitative methods in microscopy to assess pollen viability in different plant taxa. Plant Reproduction 33(3–4): 205–219. DOI: 10.1007/s00497-020-00398-6.

Ayenan M.A.T., Danquah A., Hanson P., Asante I.K., Danquah E.Y. 2021. Identification of new sources of heat tolerance in cultivated and wild tomatoes. Euphytica 217(3); 33; 16 p. DOI: 10.1007/s10681-021-02772-5.

Bosland P.W., Votava E.J. 2000. Peppers: Vegetable and Spice Capsicums. CABI, U.K., 204 p.

Driedonks N., Rieu I., Vriezen W.H. 2016. Breeding for plant heat tolerance at vegetative and reproductive stages. Plant Reproduction 29(1–2): 67–79. DOI: 10.1007/s00497-016-0275-9.

Erickson A.N., Markhart A.H. 2002. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant, Cell and Environment 25(1): 123–130. DOI: 10.1046/j.0016-8025.2001.00807.x.

Erwin A.T. 1929. A systematic study of the peppers (*Capsicum frutescens* L.). Proceedings of the American Society for Horticultural Science 26: 128–131.

Gajanayake B., Trader B.W., Reddy K.R., Harkess R.L. 2011. Screening ornamental pepper cultivars for temperature tolerance using pollen and physiological parameters. HortScience 46(6): 878–884. DOI: 10.21273/hortsci.46.6.878.

Garruña-Hernández R., Canto A., Mijangos-Cortés J.O., Islas I., Pinzón L., Orellana R. 2012. Changes in flowering and fruiting of Habanero pepper in response to higher temperature and CO_2. Journal of Food Agriculture and Environment 10(3–4): 802–808. DOI: 10.1234/4.2012.3516.

Gisbert-Mullor R., Padilla Y.G., Martínez-Cuenca M.R., López-Galarza S., Calatayud Á. 2021. Suitable rootstocks can alleviate the effects of heat stress on pepper plants. Scientia Horticulturae 290; 110529; 11 p. DOI: 10.1016/j.scienta.2021.110529.

Hedhly A., Hormaza J.I., Herrero M. 2008. Global warming and sexual plant reproduction. Trends in Plant Science 14(1): 30–36. DOI: 10.1016/j.tplants.2008.11.001.

Hirose T. 1957. Studies on the pollination of pepper. I. Flowering and pollen germination. The Scientific Reports of the Saikyo University, Faculty of Agriculture 9: 5–12. [in Japanese with English abstract]

IPCC 2014. Climate Change 2014. Synthesis Report. Intergovernmental Panel on Climate Change, Geneva, Switzerland, 151 p. https://www.ipcc.ch/site/assets/uploads/2018/02/SYR_AR5_FINAL_full.pdf (October 10, 2021)

Konisho K., Minami M., Matsushima K., Nemoto K. 2005. Phylogenetic relationship and species identification by RAPD analysis in genus *Capsicum*. Horticultural Research (Japan) 4(3): 259–264. DOI: 10.2503/hrj.4.259. [in Japanese with English abstract]

Kumazawa S., Ohara T., Niiuchi K. 1954. The differentiation of varieties of peppers in Japan. Journal of the Japanese Society for Horticultural Science 23(3): 152–158. DOI: 10.2503/jjshs.23.152. [in Japanese with English abstract]

Levy A., Rabinowitch H.D., Kedar N. 1978. Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. Euphytica 27(1): 211–218. DOI: 10.1007/bf00039137.

Lobell D.B., Asner G.P. 2003. Climate and management contributions to recent trends in U.S. agricultural yields. Science 299(5609): 1032. DOI: 10.1126/science.1078475.

Mesihovic A., Iannacone R., Firon N., Fragkostefanakis S. 2016. Heat stress regimes for the investigation of pollen thermotolerance in crop plants. Plant Reproduction 29(1–2): 93–105. DOI: 10.1007/s00497-016-0281-y.

Peng S., Huang J., Sheehy J.E., Laza R.C., Visperas R.M., Zhong X. et al. 2004. Rice yields decline with higher night temperature from global warming. Proceedings of the National Academy of Sciences 101(27): 9971–9975. DOI: 10.1073/pnas.0403720101.

R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/ (October 10, 2021)

Rani M., Jindal S.K., Vikal Y., Meena O.P. 2021. Genetic male sterility breeding in heat tolerant bell pepper: Introgression of *ms10* gene from hot pepper through marker-assisted backcrossing. Scientia Horticulturae 285; 110172; 14 p. DOI: 10.1016/j.scienta.2021.110172.

Reddy K.R., Kakani V.G. 2007. Screening *Capsicum* species of different origins for high temperature tolerance by *in vitro* pollen germination and pollen tube length. Scientia Horticulturae 112(2): 130–135. DOI: 10.1016/j.scienta.2006.12.014.

Richards A.J. 1986. Plant Breeding Systems. George Allen and Unwin, London, U.K., 529 p.

Sato S., Peet M.M., Thomas J.F. 2000. Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. Plant, Cell and Environment 23(7): 719–726. DOI: 10.1046/j.1365-3040.2000.00589.x.

Sato S., Kamiyama M., Iwata T., Makita N., Furukawa H., Ikeda H. 2006. Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development. Annals of Botany 97(5): 731–738. DOI: 10.1093/aob/mcl037.

Shirasawa K., Ishii K., Kim C., Ban T., Suzuki M., Ito T. et al. 2013. Development of *Capsicum* EST–SSR markers for species identification and *in silico* mapping onto the tomato genome sequence. Molecular Breeding 31(1): 101–110. DOI: 10.1007/s11032-012-9774-z.

Sun J.-T., Cheng G.-X., Huang L.-J., Liu S., Ali M., Khan A. et al. 2019. Modified expression of a heat shock protein gene, *CaHSP22.0*, results in high sensitivity to heat and salt stress in pepper (*Capsicum annuum* L.). Scientia Horticulturae 249: 364–373. DOI: 10.1016/j.scienta.2019.02.008.

Tubiello F.N., Soussana J.-F., Howden S.M. 2007. Crop and pasture response to climate change. Proceedings of the National Academy of Sciences 104(50): 19686–19690. DOI: 10.1073/pnas.0701728104.

Usman M.G., Rafii M.Y., Martini M.Y., Yusuff O.A., Ismail M.R., Miah G. 2018. Introgression of heat shock protein (Hsp70 and sHsp) genes into the Malaysian elite chilli variety Kulai (*Capsicum annuum* L.) through the application of marker-assisted backcrossing (MAB). Cell Stress and Chaperones 23(2): 223–234. DOI: 10.1007/s12192-017-0836-3.

Xu J., Driedonks N., Rutten M.J.M., Vriezen W.H., de Boer G.-J., Rieu I. 2017. Mapping quantitative trait loci for heat tolerance of reproductive traits in tomato (*Solanum lycopersicum*). Molecular Breeding 37(5); 58; 9 p. DOI: 10.1007/s11032-017-0664-2.

Yamazaki A., Hosokawa M. 2018. The autonomous self-pollination without pollinators in a *Capsicum chinense* F_1 hybrid. Horticultural Research (Japan) 17(Suppl. 1): 204. [in Japanese]

Yamazaki A., Hosokawa M. 2019. Increased percentage of fruit set of F_1 hybrid of *Capsicum chinense* during high-temperature period. Scientia Horticulturae 243: 421–427. DOI: 10.1016/j.scienta.2018.08.049.

Yamazaki A., Hosokawa M. 2020. Relationship between fruit-set ability of an F_1 hybrid of *Capsicum chinense* and the pollen germination rate under high temperature condition. Horticultural Research (Japan) 19(Suppl. 1): 159. [in Japanese]

Yashiro K., Sakai Y., Namai H. 1999. Relationships between pollen–ovule ratio and autofertility, self-compatibility, automatic self-pollination ability in heterogeneous incomplete autogamous plants, Thai mustard. Breeding Science 49(1): 39–42. DOI: 10.1270/jsbbs.49.39.

Zhou R., Yu X., Kjær K.H., Rosenqvist E., Ottosen C.-O., Wu Z. 2015. Screening and validation of tomato genotypes under heat stress using F_v/F_m to reveal the physiological mechanism of heat tolerance. Environmental and Experimental Botany 118: 1–11. DOI: 10.1016/j.envexpbot.2015.05.006.