Capsaicinoid Content of *Capsicum* spp. Cultivated Under High Temperature Stress

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***Abstract*—Capsaicinoid content of chili peppers plays an important role in the food industry. Environmental temperature is increasing continuously because of the greenhouse effect. High temperature is a stress for plants. Compounds like capsaicinoids and the number of seeds in fruits will change as a response to such stresses. The present experiment was conducted to determine the effect of temperature stress on capsaicinoids and the number of seeds in the 'Takanotsume', 'Habanero' and 'Himo' chili varieties. Temperature stress conditions were provided in a greenhouse and control environmental conditions were provided to the plants in an open field at the Faculty of Agriculture, Shinshu University. After harvesting fruits at 20 and 30 days after flowering (DAF), the number of seeds was counted and the capsaicinoid contents were measured using high-performance liquid chromatography. The number of seeds decreased with the temperature stress. Capsaicinoid content was significantly higher in the temperature stress condition than the control condition in all varieties except 'Himo' at 20 DAF. Altogether, an inverse relationship was observed between capsaicinoid content and the number of seeds.**

Keywords— Capsaicinoid, chili pepper, temperature stress

I. Introduction

Chili peppers are important in the food and pharmaceutical industries and are widely cultivated spices around the world. Chili pepper belongs to the genus *Capsicum* in the family Solanaceae. Capsaicinoids are the active ingredients that give chili peppers their hotness or burning taste. The interaction between the cultivar (genetic traits) and the growing environment significantly affects the accumulation of capsaicinoids in chili peppers. Drought, salinity stress, light, and fertilizer conditions are the environmental factors that affect chili pepper pungency and fruit quality. At present, the global temperature is continuously increasing and it is expected to increase by 0.5–2.8 ^{0}C at the end of the 21st century [1]. Plants also can be affected in many ways by this temperature increase, which is a stressor for plant growth and development.

There are a limited number of experiments on the effect of temperature stress on chili peppers because it is difficult to provide temperature stress without causing drought stress and changes in light conditions. On the other hand, most of the studies were mainly focused only on growth and yield parameters. The relationships of environmental stresses such as drought stress [2], salinity stress [3], and excess P fertilizer stress, with capsaicinoid and average numbers of seeds, have been already tested, except for temperature stress. Therefore, the present experiment was conducted to investigate the effect of temperature stress on capsaicinoids and seed content in chili pepper.

II. Methodology

A. Location and the Media Used in the Experiment

The experiment was carried out at Alpine Field Research and Education Centre, Faculty of Agriculture, Shinshu University, Minamiminowa, Nagano, Japan. 'Takanotsume' and 'Himo' (*C. annuum*) and 'Habanero' (*C. chinense*) varieties were used for this experiment.

The experiment was conducted from June to September, 2022. 15 cm long seedlings were transplanted into plastic pots (18 cm in diameter, 2.2 L) filled with a commercial potting medium. The first set of flower buds was removed after applying the treatments. BB fertilizer 552 (N: P: K, 15:15:12) was added following the standard recommendation.

B. Testing the Effect of Temperature

Two temperature conditions were provided during the present Experiment: a temperature stress was tested inside a greenhouse and a control experiment was conducted outside the greenhouse. The maximum average temperature was 44.4 ^{0}C, and the minimum average temperature was 14.9 ^{0}C inside the greenhouse. Outside the greenhouse, the maximum average temperature was 36.7 ^{0}C and the minimum average temperature was 13.5 ^{0}C. Similar light conditions were provided with 50% shade for both greenhouse and control tests. Excess water was provided to prevent the effect of drought. Overflow from the pot was retained in a plate under the pot and allowed to be absorbed through the pot base. Five plants were used for each experiment. One fruit from each plant was harvested at 20 and 30 DAF and stored in a -80 ˚C refrigerator until used for capsaicinoid analyses.

C. Analysis of the Capsaicinoid Content and Seed Counting

The number of seeds was counted before capsaicinoid analysis. The capsaicinoid content of the placenta septum was measured using high-performance liquid chromatography. Data were analyzed by using an Analysis of Variance (ANOVA) in Minitab 17.

III. Results and Discussion

When the number of seeds of all three varieties harvested at both 20 and 30 DAF was compared, a significantly lower average number of seeds was present in the temperature stressed condition than in the control condition. Both 'Takanotsume', and 'Habanero' showed higher capsaicinoid content during the temperature stress compared to the control at both 20 DAF and 30 DAF. Surprisingly, 'Himo' did not show a significant difference in capsaicinoid content between the temperature stress and the control at 20 DAF (Tab. 1).

However, significantly higher capsaicinoid content was reported in the temperature stress than the control condition at 30 DAF. When the capsaicinoid contents that were reported during the same treatment and the same variety were compared based on harvesting times, no significant difference was shown in 'Takanotsume' and 'Habanero'. However, in 'Himo', fruits harvested at 30 DAF showed higher capsaicinoid content in the temperature stress condition than fruits harvested at 20 DAF in the temperature stress treatment. Among all the treatments, the highest capsaicinoid content was reported in 'Habanero' during the temperature stress condition harvested at 30 DAF, whereas the lowest was reported in 'Himo' which was grown under control conditions, harvested at 20 DAF.

The pungency or the hotness which is considered as the main characteristic of the chili fruits, increases when the amount of capsaicinoid is increased. An inverse relationship can be observed between the average number of seeds and the capsaicinoid content in all varieties at all harvesting stages. Under the temperature stress condition, capsaicinoid content was increased when the average number of seeds was decreased.

During the seed formation period of chili peppers, exposure to high temperatures after anthesis, severely affects the fruit growth and decreases the number of seeds per fruit in 'Shishito' peppers [4]. The biochemical pathways of the production of lignin and capsaicin are linked where the production of one compound adversely affects the production of the other compound, resulting in a trade-off between the two compounds [5]. Lignin is a major structural compound that is associated with the seed coat. The need to produce lignin with the reduction of seed number becomes less, providing space to produce high capsaicinoids in a trade-off scenario. This phenomenon could be possible and is the most suitable explanation for the higher amount of capsaicinoid content reported during the temperature stress condition in the present study. However, the 'Himo' variety was an exception with regard to the capsaicinoid content reported during the stress conditions. In the case of 'Himo' pepper, HPLC could not detect a sufficient amount of capsaicinoid at 20 DAF to support the results of other varieties.

IV. Conclusion

Since the capsaicinoid content increases, the pungency was enhanced when temperature stress was applied. As a suggestion, if farmers use temperature stress conditions by growing in a greenhouse, rather than growing plants at the normal environmental temperature, they can get more pungent chili fruits.

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TABLE 1. CAPSAICINOID CONTENT AND AVERAGE NUMBER OF SEEDS OF 'TAKANOTSUME', 'HABANERO' AND 'HIMO' GROWN UNDER DIFFERENT TEMPERATURE TREATMENTS IN 2022

Varieties	Temperature tretment	Capsaicinoid content of placenta septum (µg·g-1 DW)		Average number of seeds	
		Harvesting date		Harvesting date	
		20 DAF	30 DAF	20 DAF	30 DAF
Takanotsume	High T-Stress	45242 ± 5710 a	49170 ± 9474 x	28.8 ± 4.2 d	30.6 ± 1.9 q
	Control	29623 ± 3699 b	34063 ± 4085 y	37.0 ± 3.4 c	38.0 ± 2.7 p
Habanero	High T-Stress	102850 ± 2419 a	126980 ± 17304 x	18.8 ± 2.6 d	23.2 ± 1.7 q
	Control	78545 ± 3065 b	93358 ± 9271 y	26.4 ± 2.2 c	30.6 ± 2.2 p
Himo	High T-Stress	795 ± 91 a	2469 ± 241 x	28.0 ± 4.5 d	28.2 ± 2.4 q
	Control	597 ± 85 a	597 ± 350 y	36.6 ± 3.6 c	37.0 ± 2.8 p

Values (means ± standard deviation, n=5) followed by the different letters (a, b, x, y, d, c, p and q) in a column of each temperature treatment are significantly different at 5% level by Tukey's pairwise test.