Relationship between the soluble solids metric Brix and other traits in *Capsicum* peppers

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Abstract

The present study was conducted to clarify the relationship between fruit Brix and other chemical components related to taste in chili peppers and to clarify how Brix, which changes in response to environmental stress, is related to other traits such as yield. The experiments were conducted from 2015 to 2019. In experiment 1, 39 varieties of *Capsicum annuum* were cultivated in 2015 and their pungency and the taste components were evaluated. Relationship of pungency and taste components among varieties were also investigated. In experiment 2, Japanese chili pepper cultivars 'Manganji', 'Fushimiamanaga' and 'Botankosho' were grown under several stress conditions from 2015 to 2019 to determine the relationships of pungency and the taste components in each individual variety. In experiment 3, we observed the effect of stress conditions on plant growth performance and yield using drought stress and salinity stress on 'Shishito', 'Sapporo' and 'Botankosho' cultivars. In experiment 1, although significant positive correlation was found between total sugar content and glucose content in all groups, there was no significant positive correlation between Brix and total sugar content. In experiment 2, a significant positive correlation was found between total sugar content and Brix in all cultivars under all stress conditions. Moreover, a significant positive correlation was found between total sugar content and Brix in each variety. These results clarified that it was not appropriate to use Brix for the comparison of varietal difference of total sugar content but that it was possible to estimate the differences among treatments and individuals within the same variety. In experiment 3, we also found that stress during cultivation increased Brix and capsaicinoid content, and decreased yield, fruit size, and number of leaves. The stress induced increase in Brix because of reduced yield and fruit size, and the effect of reduced photosynthetic ability on Brix due to reduced number of leaves seemed rather small.

Key words: Brix, Capsaicinoid, *Capsicum annuum*, total soluble solid, total sugar

Introduction

Chili peppers belong to the genus *Capsicum* in the Solanaceae family, and are an important source of spices because their capsaicinoids give a sensation of hotness when eaten. Chili peppers are popular as vegetables (fresh or cooked) as well as spices in most parts of the world, and thus it is important to

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maintain fruit quality to supply the best product. Many physiological factors affect the quality of the fruit, such as size, shape, and color. In addition, flavor, aroma, nutrient content, and taste components (sugar, glutamic acid, Brix, etc.) are also important factors. The perception of flavor is influenced by many factors, and taste (sweetness, sourness) is determined basically by sugars and acids[9].

Soluble solid content is an important quality parameter, both as a sensory flavor and as a processing requirement[2]. Also, when measuring the total sugar and glucose contents at the farm level, most farmers measure those using Brix. However, Brix actually indicates total soluble solids (TSS) in the fruit. Fruits consist of many water-soluble components, such as glucose, sucrose, fructose, and water-soluble proteins. In cultivated tomatoes, the soluble (SS) and insoluble solids (ISS) account for about 75% and 25%, respectively, of the total solids[10]. Reducing sugars (glucose and fructose) are the major components of the SS; sucrose is also present but in very small quantities[12]. This means that Brix indicates not only total sugar and glucose, but also all of the dissolved components in fruits. Therefore, it is debatable whether Brix is an appropriate measure of sugar. On the other hand, there may be a relationship between Brix (TSS) and total sugar content, if reducing sugars play a major role in TSS.

Moreover, stress conditions such as water- and salinity-stress can increase the contents of taste components in chili pepper fruits[16, 17]. The productivity of chili peppers is also often reduced by both biotic and abiotic stresses, the types of which can vary among regions[1]. Changes in climatic factors, such as temperature, precipitation, and the frequency and severity of extreme weather events, such as drought, floods and windstorms, directly affect crop yields. Plant growth, development, and yield of chili peppers is also influenced by climate change. It is thus also important to discover the effects of environmental stresses (water/salinity) on Brix, plant growth parameters, and yields in chili peppers.

The present study was conducted to clarify the relationship between Brix and chemical components related to taste, such as sugars, in *Capsicum* peppers. We further examined how Brix, which changes in response to environmental stress, is related to other traits such as crop yield.

Materials and Methods

Plant materials and experimental design

Experiment 1

On 29 March 2015, seeds were sown and germinated, and 39 strains were planted on 11 June 2015 at the experimental farm (773 m a.s.l.) of the Education and Research Center of Alpine Field Science (AFC), Faculty of Agriculture, Shinshu University in Minamiminowa, Nagano, Japan.

Among these strains, 28 varieties of *C. annuum* were purchased from a domestic seed company, nine strains/varieties were gerplasms preserved by Plant Breeding and Genetics Laboratory of Shinshu University, one variety was a preserved variety transferred from a gene bank, National Agriculture and Food Research Organization, and one variety was transferred from other university. The details of the tested varieties and strains are shown in Table 1. In this research, varieties and strains were classified as 'Sweet Pepper/Paprika', 'Sweet/Very low pungent chili pepper', and 'Pungent chili pepper/Chili pepper'.

The strains/varieties used for the experiments were cultivated at the experimental farm using five individuals per strain. In the experiments, the planted areas used ridges (raised beds) with a width of 75 cm, and the width between ridges was 100 cm. Ridges were covered with a black polythene mulch and planted in a single row with spacing of 40 cm. BB fertilizer 552 (N:P:K, 15:15:12) was added at 20 kg/acre, and no additional fertilizer was applied after planting. Admire (Bayer Crop science) was appropriately sprayed on the stock as a countermeasure against pests and diseases. Fruits harvested from each strain were used for the analysis of glucose, total sugar (glucose and fructose), glutamic acid content, Brix percentage, and capsaicinoid content.

Table 1. Test materials for experiment 1 in 2015. Varieties and strains are classified as 'Sweet pepper/Paprika', 'Sweet/Very low pungent chili pepper', and 'Pungent chili pepper/Chili pepper'.

Varieties or Strain names	Seed company and Production area
Sweet pepper/ Paprika	
Sweet pepper 1	Seed company 1
Sweet pepper 2	Seed company 1
Sweet pepper 3	Seed company 3
California Wonder 1	Seed company 4
California Wonder 2	Seed company 5
Sweet pepper 4	Seed company 6
Sweet pepper 5	Seed company 1
Paprika 1	Seed company 1
Paprika 2	Seed company 7
Paprika 3	Seed company 8
Sweet/ Very low pungent chili pepper	
Ise Piman	Seed company 6
Fushimi ama naga 1	Seed company 1
Fushimi ama naga 2	Seed company 2
Manganji 1	Seed company 4
Manganji 2	Seed company 6
Manganji type	Seedc ompany 8
Sanpou Ooamanago	Tottori Prefecture (Gene bank JP32567)
Pungent chili pepper / Chili pepper	
Botankosho type 1	Seed company 9
Botankosho type 2	Seed company 9
Takanotsume 1	Seed company 1
Takanotsume 2	Seed company 2
Shishitou 1	Seed company 1
Shishitou 2	Seed company 2
Sapporo Namban 1	Seed company10
Kagura Namban 1	Seed company11
Kagura Namban 2	Seed company12
Kagura Namban 3	Seed company 6
Botankosho 1	Seed company 5
Sapporo Namban 2	Seed company 5
Botankosho 2	Nakano, Nagano prefecture
Hishino Nanban 1	Komoro, Nagano prefecture
Hishino Nanban 2	Komoro, Nagano prefecture
Suzugasawa Nanban	Anan, Nagano Perfecture
Local chili pepper Nagano prefecture 1	Nagano Prefecture
Shishi Kosho	Sakae, Nagano Prefrcture
Local chili pepper Nagano prefecture 2	Nagano Prefecture
Local chili pepper Nagano prefecture 3	Nagano Prefectue
Takato Tentou Nanban	Ina, Nagano Prefectue
Shimizumori namba	Hirosaki, Aomori Prefecture (Hirosaki University)

Experiment 2

The experiment was carried out from 2015 to 2019 in a greenhouse at the experimental farm (773 m a.s.l.) of AFC, Faculty of Agriculture, Shinshu University in Minamiminowa, Nagano, Japan. Local Japanese chili pepper cultivars 'Manganji' and 'Fushimiamanaga' were used in the experiment. Both cultivars are non-pungent vegetable cultivars that originated in Kyoto Prefecture. Seeds of 'Fushimiamanaga' were purchased from Takii Seed Co. Ltd. (Kyoto, Japan), and seeds of 'Manganji' were purchased from Noguchi Seeds (Saitama, Japan). In addition, the local pungent variety 'Botankosho', which is used as a traditional vegetable in northern Nagano Prefecture, was grown from seeds donated by the Madarao Botankosho Conservation Society (Nakano, Japan). The nursery period was from the end of March to mid-April each year. The seedlings were grown in a greenhouse in 75-mm plastic pots filled with a commercial potting medium (Nae-ichiban, Sumitomo Forestry Landscaping Co., Ltd., Tokyo, Japan). During the seedling rearing period, the greenhouse was heated using oil heaters at night until early April to keep the temperature above 15° C. To analyze the relationship with taste components, we used several kinds of stress conditions. As stress treatments, we applied three volumes of water in individual applications to represent drought (D; 50 mL water per application), standard water supply (S; 130 mL water per application), and excess water supply (E; 260 mL water per application). We applied three salinity levels using sodium chloride (NaCl; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan): excessive salinity (E; 10 dS/m (6.4 g/L)), additional salinity (A; 5 dS/m (3.2 g/L)), and normal salinity control (C; 0.9 dS/m (0.57 g/L)). A randomized complete block design was used for the experiments.

During the cultivation period, a slow-acting home gardening fertilizer (N:P:K, 10:10:10; Shizen Oyokagaku Co. Ltd., Nagoya, Aichi, Japan) was once applied in August every year. The water was appropriately applied considering the daily temperature and weather as follows. On sunny days and/or when the daily temperature exceeded 30° C, water was applied three times per day. On rainy or cloudy days and/or when the daily temperature was lower than 30° C, water was applied twice per day. Throughout the experiment, plants were grown in one green house and other management practices were applied equally to all plants.

During the stress treatment, each flower was tagged at the end of anthesis to ensure that fruit could be harvested at 40 days after flowering (DAF). The harvested fruits were stored at -80° C until analysis.

Fruits harvested for each stress treatment were used to analyze glucose, total sugar (glucose and fructose), glutamic acid content, Brix percentage, and capsaicinoid content. Six individuals of each cultivar applied with each treatment were used for each treatment. Three samples each consists of 50 g of fruit (approximately 1–6 fruits) were chosen randomly for the analysis of taste components. The minimum fruit tissue weight necessary for spectrophotometric analysis was 50 g. This practice was continued in each year to analyze the taste components.

Experiment 3

The experiment was conducted from April to October 2020 in a greenhouse at the experimental farm (773 m a.s.l.) of AFC, Faculty of Agriculture, Shinshu University in Minamiminowa, Nagano, Japan. Water stress and salinity stress treatments and other practices were the same in experiment 2. For water stress treatments, we used three Japanese cultivars of chili pepper, 'Shishito' (seeds purchased from Takii Seed Co., Ltd. Kyoto, Japan); the local pungent variety 'Botankosho', which is used as a traditional vegetable in northern Nagano Prefecture (seeds donated by the Madarao Botankosho Conservation Society (Nakano, Nagano, Japan)); and 'Sapporo Oonaga Nanban' ('Sapporo'; Tsurushin Seed, Matsumoto, Japan), which is a local variety originating in Hokkaido (Fig. 1). For salinity stress treatments, we used 'Shishito' and 'Sapporo' cultivars.

Before treatments, seedlings of approximately 150 mm in height were transplanted to plastic pots

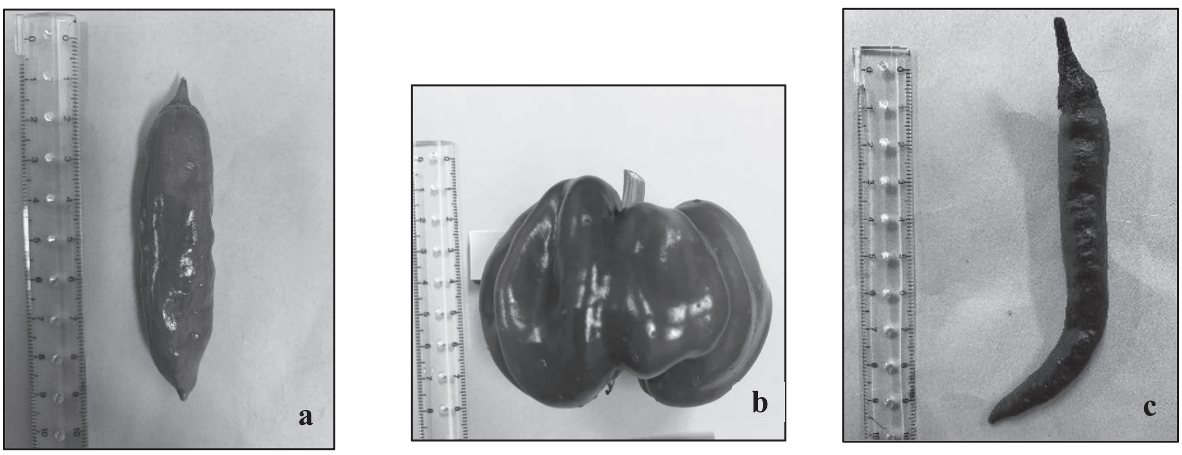

Fig. 1. Fruits of Japanese chili pepper (*Capsicum annuum*) cultivars included in this study: (a) 'Shishito' (Takii), '(b) Botankosho' (Local), and '(c) Sapporo Oonaga Nanban' (Tsurushin).

(diameter 18 cm, volume 1.8 L) filled with 1 kg of the same commercial potting medium as that used in experiment 2, and the stress treatments were applied starting 1 week after the seedlings were transplanted. All flowers including flower buds were removed before applying stress treatments. During the stress treatment, each flower was tagged at the end of anthesis to ensure that the fruit could be harvested at around 30 DAF. Fruits harvested for each treatment were used for measuring Brix and capsaicinoid contents in the placental septum at minimum and maximum stress conditions. In addition, data was collected as harvested number of fruits, fruit weight, total yield, number of leaves, number of branches, and plant height were measured on a per plant basis. A randomized complete block design was used for the analysis of experimental data.

Solution preparation for analysis of sugar and glutamic acid contents

Extracts for sugar and glutamic acid analyses were prepared from a known amount of ground fruit tissue obtained by using a grinder (YMB-400, Yamazen, Osaka, Japan) and filtered with No.1, 125-mm filter paper (ADVANTEC®, Tokyo, Japan). Extracts were prepared for quantitative analysis of glucose, total sugar (glucose + fructose), and glutamic acid using a digital portable spectrophotometer (RQflex 10 plus, Merck, Darmstadt, Germany). According to Nonaka et al.[13], glucose, total sugar, and glutamic acid contents in sweet pepper fruits detected by a RQflex spectrophotometer and by capillary electrophoresis methods[7], yielded similar results.

Solution preparation for analysis of Brix

Brix is primarily a measure of the sugar concentration in a solution. Extracts were prepared from ground fruit tissues obtained by using a grinder (YMB-400, Yamazen) followed by filtering through No.1, 125-mm filter paper (ADVANTEC®). Extracts were used directly to measure Brix with a digital portable refractometer (Pen-J, Atago Co. Ltd., Tokyo, Japan).

Capsaicinoid analysis using HPLC

The HPLC analysis conditions were as follows: LC column (50 × 3.0 mm; Shimadzu Corporation, Kyoto, Japan), column temperature 40° C, mobile phase 70% methanol, flow rate 1 mL/min, and absorbance at 280 nm. To validate the analytical conditions, capsaicin (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as a standard. Standard capsaicin solutions of 62.5, 125, 250, and 500 μg/mL[14] were analyzed, and a calibration curve was prepared. Sample solutions were prepared using freeze-dried chili pepper for 24h, and capsaicin was extracted after ground chili pepper powder (2 mg) was added to methanol (20 mL) and being kept for 1 h at 40° C.

Results

Experiment 1

1. Brix

Table 2 shows the results of Brix measurement. The lowest Brix was 6.57% for ‘California wonder 1’ and the highest was 10.03% for ‘Sweet pepper 1’ among the ‘Sweet pepper/Paprika’ group, which had an average of 8.26%. The ‘Sweet/Very low pungent chili pepper’ group had an average of 10.79% Brix, with 7.07% the lowest value for ‘Sanpou Ooamanago’ and 13.03% the highest value for ‘Fushimiamanaga 2’. In the ‘Pungent chili pepper/Chili pepper’ group, the average was 10.87% Brix, with 6.93% the lowest value for ‘Botankosho type 1’ and 16.30% the highest value for ‘Local chili pepper Nagano prefecture 1’. Among the all cultivars used in this experiment the lowest Brix was from ‘California wonder 1’ and the highest was from ‘Local chili pepper Nagano prefecture 1’.

2. Glucose content

The results are shown in Table 2. The lowest glucose content of 1834 mg/100 g (FW) was recorded for 'Sweet pepper 4' and the highest of 2931 mg/100 g (FW) was for 'Sweet pepper 5' among the 'Sweet pepper/Paprika' group, which had an average of 2169 mg/100 g (FW). The 'Sweet/Very low pungent chili pepper' group had an average of 2042 mg/100 g (FW) glucose content, with 968 mg/100 g (FW) as the lowest for 'Sanpou Ooamanago' and 3122 mg/100 g (FW) as the highest for 'Manganji 1'. The 'Pungent chili pepper/Chili pepper' group had an average of 1953 mg/100 g (FW) glucose content, with 1090 mg/100 g (FW) as the lowest for 'Takanotsume 1' and 3040 mg/100 g (FW) as the highest for 'Sapporo Namban 2'. Among the all cultivars used in this experiment, the lowest glucose content was in 'Sanpou Ooamanago' and the highest was in 'Manganji 1'.

3. Total sugar content

Table 2 shows the results of total glucose and fructose contents. The lowest total sugar content of 4154 mg/100 g (FW) was recorded for 'Sweet pepper 4' and the highest of 5466 mg/100 g (FW) was for 'Sweet pepper 5' among the 'Sweet pepper/Paprika' group, which had an average of 4735 mg/100 g (FW). The 'Sweet/Very low pungent chili pepper' group had an average of 5074 mg/100 g (FW) total sugar content, with 3101 mg/100 g (FW) the lowest for 'Sanpou Ooamanago' and 7571 mg/100 g (FW) the highest for 'Manganji 1'. The 'Pungent chili pepper/Chili pepper' group had an average of 4415 mg/100 g (FW) total sugar content, with 2784 mg/100 g (FW) the lowest for 'Takanotsume 1' and 6385 mg/100 g (FW) the highest for 'Sapporo Namban 2'. Among the all cultivars used in this experiment, the lowest total sugar content was in 'Takanotsume 1' and the highest was in 'Manganji 1'.

4. Glutamic acid content

Table 2 shows the glutamic acid contents. The lowest glutamic acid content of 36.7 mg/100 g (FW) was recorded for 'Sweet pepper 2' and the highest of 78.5 mg/100 g (FW) was for 'Sweet pepper 5' among the 'Sweet pepper/Paprika' group, which had an average of 61.6 mg/100 g (FW). The 'Sweet/Very low pungent chili pepper' group had an average of 72.2 mg/100 g (FW) glutamic acid) content, with 0.0 mg/100 g (FW) the lowest for 'Fushimiamanaga 1' and 108.1 mg/100 g (FW) the highest for 'Manganji 1'. The 'Pungent chili pepper/Chili pepper' group had an average of 42.9 mg/100 g (FW) glutamic acid content, with 5.1 mg/100 g (FW) the lowest for 'Local chili pepper Nagano prefecture 3' and 85.6 mg/100 g (FW) the highest for 'Botankosho type 1'. Among the all cultivars used in this experiment, the lowest glutamic acid content was in 'Local chili pepper Nagano prefecture 3' and the highest was in 'Manganji 1'.

5. Capsaicinoids

The results of capsaicinoid content are shown in Table 2. The 'Sweet/Very low pungent chili pepper' group did not have any capsaicinoid content. The 'Pungent chili pepper/Chili pepper' group had the highest total capsaicinoid content of 2128 μg/g (DW) for 'Takato Tentou Nanban', and the lowest total capsaicinoid content was 81.5 μg/g (DW) for 'Botankosho type 2', with the average being 496.8 μg/g (DW).

6. Correlation analysis

The correlation coefficients for each item are shown in Table 3a-d. A significant positive correlation at the 1% level was found between the total sugar content and glucose content in the 'Sweet pepper/Paprika' group in terms of content per fresh fruit weight. In the 'Sweet/Very low pungent chili pepper' group, significant positive correlations were found between total sugar content and glucose content per fresh fruit weight at the 1% level, and between glutamic acid content and total sugar content per fresh fruit weight at the 5% level.

Table 2. Brix, taste components, and capsaicinoid (CAP) in varieties and lines used for experiment 1.

Varitety or strain name	Moisture content (%)	Brix (%)	Glucose (mg.100 g^{-1} FW)	Total sugar (mg.100 g^{-1} FW)	Glutamic Acid (mg.100 g^{-1} FW)	CAP (μg/g . DW)
Sweet pepper/ Paprika						
Sweet pepper 1	86.0	10.03 ± 0.34	2282 ± 155	5125 ± 331	50.2 ± 8.4	-
Sweet pepper 2	88.5	8.70 ± 0.00	1894 ± 89	4160 ± 53	36.7 ± 4.9	-
Sweet pepper 3	89.9	7.77 ± 0.17	2146 ± 61	4987 ± 196	39.9 ± 1.5	-
California Wonder 1	90.4	6.57 ± 0.05	2086 ± 61	4389 ± 380	53.4 ± 5.9	-
California Wonder 2	88.4	8.80 ± 0.00	1945 ± 0	4809 ± 0	59.2 ± 0.0	-
Sweet pepper 4	89.9	8.70 ± 0.80	1834 ± 144	4154 ± 342	58.4 ± 30.9	-
Sweet pepper 5	87.6	8.63 ± 0.12	2931 ± 17	5466 ± 301	78.5 ± 9.1	-
Paprika 1	89.8	7.50 ± 0.08	2430 ± 74	5303 ± 455	71.8 ± 1.9	-
Paprika 2	88.8	7.97 ± 0.17	2403 ± 165	4581 ± 523	62.4 ± 12.1	-
Paprika 3	83.3	7.03 ± 0.34	2011 ± 253	4689 ± 898	76.9 ± 7.9	-
Sweet/ Very low pungent chili pepper						
Ise Piman	83.3	9.13 ± 0.09	1899 ± 54	4420 ± 355	80.0 ± 14.7	-
Fushimi ama naga 1	82.2	11.50 ± 0.00	1709 ± 0	3911 ± 0	18.1 ± 0.0	-
Fushimi ama naga 2	78.9	13.03 ± 0.17	2052 ± 112	4820 ± 405	96.6 ± 8.2	-
Manganji 1	85.1	11.80 ± 0.24	3122 ± 354	7571 ± 937	108.1 ± 11.4	-
Manganji 2	83.4	11.93 ± 0.74	2368 ± 82	5771 ± 563	62.3 ± 8.9	-
Manganji type	86.1	11.10 ± 0.22	2182 ± 16	5926 ± 250	106.8 ± 23.2	-
Sanpou Ooamanago	87.4	7.07 ± 0.25	968 ± 141	3101 ± 512	33.8 ± 6.7	-
Pungent chili pepper/ Chili pepper						
Botankosho type 1	89.2	6.93 ± 0.09	2200 ± 17	4947 ± 370	85.6 ± 3.2	0.0 ± 0.0
Botankosho type 2	87.9	8.63 ± 0.17	2474 ± 72	5628 ± 274	76.2 ± 12.2	81.5 ± 28.5
Takanotsume 1	69.8	14.57 ± 0.09	1090 ± 105	2784 ± 184	51.2 ± 6.7	1392.9 ± 17.6
Takanotsume 2	69.8	15.17 ± 1.53	1182 ± 57	3089 ± 414	60.0 ± 9.9	966.0 ± 6.7
Shishitou 1	80.5	10.63 ± 0.26	1288 ± 70	3627 ± 646	42.4 ± 16.2	0.0 ± 0.0
Shishitou 2	81.8	11.20 ± 0.08	2115 ± 202	5679 ± 576	44.4 ± 0.8	0.0 ± 0.0
Sapporo Namban 1	76.6	13.20 ± 0.00	1623 ± 0	4472 ± 0	32.2 ± 0.0	775.2 ± 7.8
Kagura Namban 1	85.1	8.27 ± 0.77	1952 ± 201	4710 ± 264	31.5 ± 4.3	0.0 ± 0.0
Kagura Namban 2	89.2	7.73 ± 0.12	2925 ± 50	5719 ± 641	41.6 ± 2.8	0.0 ± 0.0
Kagura Namban 3	85.5	7.90 ± 0.28	1904 ± 58	4025 ± 129	11.1 ± 6.0	135.2 ± 28.2
Botankosho 1	88.1	8.37 ± 0.31	1950 ± 44	4888 ± 432	73.7 ± 9.0	150.6 ± 63.7
Sapporo Namban 2	78.8	10.40 ± 0.14	3040 ± 47	6385 ± 277	5.8 ± 2.9	628.9 ± 7.0
Botankosho 2	88.1	10.10 ± 0.00	2009 ± 0	5463 ± 0	66.1 ± 0.0	1016.4 ± 9.3
Hishino Nanban 1	88.0	8.37 ± 0.31	2183 ± 76	4882 ± 191	67.2 ± 16.1	355.4 ± 4.3
Hishino Nanban 2	88.7	7.85 ± 0.15	1596 ± 0	3529 ± 231	31.9 ± 5.3	379.4 ± 3.3
Suzugasawa Nanban	76.8	11.03 ± 0.17	1783 ± 130	4016 ± 195	16.9 ± 2.7	1321.9 ± 2.7
Local chili pepper Nagano prefecture 1	69.3	16.30 ± 0.64	1673 ± 69	3272 ± 329	64.0 ± 6.5	1120.4 ± 5.6
Shishi Kosho	79.4	11.53 ± 0.41	2849 ± 79	5857 ± 285	14.0 ± 3.8	166.4 ± 5.2
Local chili pepper Nagano prefecture 2	81.6	10.85 ± 0.25	1371 ± 0	2889 ± 571	6.5 ± 0.8	0.0 ± 0.0
Local chili pepper Nagano prefecture 3	76.2	12.97 ± 0.26	2104 ± 43	4178 ± 583	5.1 ± 1.0	181.4 ± 7.5
Takato Tentou Nanban	73.0	13.80 ± 0.43	1753 ± 149	4032 ± 95	55.0 ± 12.6	2128.0 ± 16.7
Shimizumori namba	77.0	13.30 ± 0.00	1910 ± 0	3080 ± 0	63.1 ± 0.0	131.8 ± 26.5

Values are means ± SE. n = 3

Table 3a. Correlation coefficients for the 'Sweet pepper/Paprika' group in experiment 1, 2015.

	Brix	Glucose	Total sugar
Brix	–		
Glucose	0.066	–	
Total sugar	0.157	0.789**	–
Glutamic acid	−0.250	0.528	0.460

** represents the significant level at 1%, * represents the significant level at 5%
n=10

Table 3b. Correlation coefficients for the 'Sweet/ Very low pungent chili pepper' group in experiment 1, 2015.

	Brix	Glucose	Total sugar
Brix	–		
Glucose	0.695	–	
Total sugar	0.590	0.968**	–
Glutamic acid	0.420	0.704	0.755 *

** represents the significant level at 1%, * represents the significant level at 5%
n=7

Table 3c. Correlation coefficients for the 'Pungent chili pepper/Chili pepper' group in experiment 1, 2015.

	Brix	Glucose	Total sugar	Glutamic acid
Brix	–			
Glucose	−0.446*	–		
Total sugar	−0.505*	0.875**	–	
Glutamic acid	−0.029	−0.117	0.001	–
Capsaicinoid	0.602**	−0.345	−0.288	−0.104

** represents the significant level at 1%, * represents the significant level at 5%
n=22

Table 3d. Correlation coefficients for all cultivars in experiment 1, 2015.

	Brix	Glucose	Total sugar
Brix	–		
Glucose	−0.250	–	
Total sugar	−0.237	0.876**	–
Glutamic acid	−0.030	0.222	0.352

** represents the significant level at 1%, * represents the significant level at 5%
n=39

In addition, in the 'Pungent chili pepper/Chili pepper' group, strong positive correlations at the 1% level between total sugar content and glucose content, and between capsaicinoid content and Brix, were found in terms of content per fresh fruit weight. Also, significant negative correlations were found between glucose content and Brix, and total sugar content and Brix, at the 5% level. When accounting for all cultivars used in 2015, a significant positive correlation at the 1% level was found between total sugar content and glucose content both in terms of content per fresh fruit weight.

Experiment 2

1. Correlation analysis of 'Manganji'

The correlation coefficients for 'Manganji' are shown in Table 4a. A significant positive correlation at the 5% level was found between glucose content and Brix. Also, significant positive correlations at the 1% level were found between total sugar content and Brix, and glutamic acid content and Brix. In addition, a significant positive correlation at the 5% level in terms of content per fresh fruit weight was found for glutamic acid content and glucose content. A significant positive correlation at the 1% level was also found between glutamic acid content and total sugar content in terms of content per fresh fruit weight.

2. Correlation analysis of 'Fushimiamanaga'

The correlation coefficients for 'Fushimiamanaga' are shown in Table 4b. A significant positive correlation at the 5% level was found between total sugar content and Brix. In addition, significant negative correlations at the 5% level were found between glutamic acid content and glucose content, and glutamic acid content and total sugar content, in terms of content per fresh fruit weight.

3. Correlation analysis of 'Botankosho'

The correlation coefficients for 'Botankosho' are shown in Table 4c. A significant positive correlation at the 1% level was found between total sugar content and Brix.

Experiment 3

1. Plant height

Plant height of all cultivars tended to decrease with increasing drought or salinity stress. In 'Shishito' and 'Sapporo', a significant difference in the plant height was seen between excess water stress and drought stress conditions (Table 5). Significant differences in plant height were seen between all water stress conditions in 'Botankosho'. The lowest plant heights were observed in drought stress conditions in all cultivars. Salinity stress-treated plants behaved the same as those under water stress (Table 6). In 'Shishito', significant differences in plant height were seen between all salinity stress treatments. A significant difference in plant height was seen between control and excess salinity stress treatment in 'Sapporo'. Correlation between Brix and total sugar content was not found in either cultivar group 'Sweet

Table 4a. Correlation coefficients for 'Manganji' in experiment 2, 2015-2019.

	Brix	Glucose	Total sugar
Brix	–		
Glucose	0.369	–	
Total sugar	0.827**	0.315	–
Glutamic acid	0.750**	0.574*	0.719**

** represents the significant level at 1%, * represents the significant level at 5%
n=14

Table 4b. Correlation coefficients for 'Fushimiamanaga' in experiment 2, 2015-2019.

	Brix	Glucose	Total sugar
Brix	–		
Glucose	−0.142	–	
Total sugar	0.515*	0.162	–
Glutamic acid	−0.104**	−0.777*	−0.361*

** represents the significant level at 1%, * represents the significant level at 5%
n=14

Table 4c. Correlation coefficients for 'Botankosho' in experiment 2, 2015-2019.

	Brix	Glucose	Total sugar	Glutamic acid
Brix	–			
Glucose	−0.213	–		
Total sugar	0.711**	−0.001	–	
Glutamic acid	0.319	−0.170	0.290	–
Capsaicinoid	0.314**	0.132	0.121	0.355

** represents the significant level at 1%, * represents the significant level at 5%
n=14

pepper/ Paprika'.

2. Number of branches

The number of branches of all cultivars tended to decrease with increasing drought or salinity stress. In 'Shishito', significant differences in the number of branches were seen between all water stress treatments (Table 5). A significant difference in the number of branches was seen between excess water stress and drought stress conditions in 'Sapporo' and 'Botankosho'. The lowest numbers of branches were observed in drought stress conditions in all cultivars. Salinity stress-treated plants behaved the same as those under water stress (Table 6). A significant difference in the number of branches was seen between control and excess salinity stress conditions in 'Shishito' and 'Sapporo'.

3. Number of leaves

The number of leaves of all cultivars tended to decrease with increasing drought or salinity stress. Significant differences in the number of leaves were seen between all water stress treatments in 'Shishito' and 'Botankosho'. In 'Sapporo', a significant difference in the number of leaves was seen between excess water stress and drought stress treatments (Table 5). The lowest numbers of leaves were observed in drought stress conditions in all cultivars. Salinity stress-treated plants behaved the same as those under water stress (Table 6). Significant differences in the number of leaves were seen between all salinity stress treatments in 'Shishito'. In 'Sapporo', a significant difference in the number of leaves was seen between control and excess salinity stress treatment.

4. Number of fruits

The number of fruits in all cultivars tended to decrease with increasing drought or salinity stress. Significant differences in the number of fruits were seen between all water stress treatments for all cultivars (Table 5). The lowest number of fruits was observed under drought stress conditions in all cultivars. Salinity stress-treated plants behaved the same as those under water stress (Table 6). Significant differences in the number of fruits were seen between all salinity stress treatments in 'Shishito' and 'Sapporo'.

5. Fruit weight

The fruit weights of all cultivars tended to decrease with increasing drought or salinity stress. A significant difference in fruit weight was seen between excess water stress and drought stress conditions in 'Shishito' and 'Sapporo'. In 'Botankosho', significant differences in fruit weight were seen between all water stress treatments (Table 5). The lowest fruit weights were observed in the drought stress condition for in all cultivars. Salinity stress-treated plants behaved the same as the water stress-treated plants (Table 6). Significant differences in fruit weight were seen between all salinity stress treatments in 'Shishito' and 'Sapporo'.

6. Total yield

The total yield of all cultivars tended to decrease with increasing stress drought or salinity stress. Significant differences in the total yield were seen between all water stress treatments in 'Shishito' and 'Botankosho'. In 'Sapporo', a significant difference in total yield was seen between excess water stress and drought stress conditions (Table 5). The lowest total yields were observed in drought stress conditions in all cultivars. Salinity stress-treated plants behaved the same as those under water stress (Table 6). A significant difference in the total yield was seen between all salinity stress treatments in 'Shishito' and 'Sapporo'.

7. Brix

The Brix in the fruits of all cultivars tended to increase with increasing drought or salinity stress in the soil. Significant differences in Brix were seen between all water stress treatments and all cultivars in water stress treatment experiment (Table 5). The highest Brix was observed in the drought stress treatment in all cultivars. Salinity stress-treated plants behaved the same as the water stress-treated plants (Table 6). Significant differences in Brix were seen between all salinity stress treatments in 'Shishito', and a significant difference was observed between excess salinity stress treatment and control treatment in 'Sapporo'.

8. Capsaicinoids in placental septum

Capsaicinoid contents in placental septum significantly increased under drought stress conditions and excess salinity stress condition in 'Shishito' and 'Sapporo' (Fig. 2).

Discussion

The results of experiment 1 showed that pepper cultivars, including native Japanese cultivars, varied greatly in their taste component content, indicating diversity within the species. This is thought to be the result of differentiation into various varieties due to differences in their uses.

Brix is an easy method of measuring sugar content for the selection of better products or for future selection of breeding parents. However, in terms of the content per fresh fruit weight, no correlation was found between Brix and total sugar content both in 'Sweet pepper/Paprika' and 'Sweet/Very low pungent chili pepper' groups when all cultivars were measured as a group. This indicates Brix is not proper indicator of total sugar content of cultivars belonging to one of the two groups. However, in 'Pungent chili pepper/Chili pepper' group, there was a positive correlation between Brix and total sugar content.

Brix is an index of the content of all soluble components regardless of taste since the concentration is measured by the refraction of light. Therefore, as the density of soluble solids in the liquid increases, the refractive index also increases proportionally, yet sugar is not always responsible for soluble solid content.

Table 5. Effect of water stress for plant growth parameters, pod parameters, yield per plant and Brix in 'Shishito' 'Sapporo Oonaga Nanban' and 'Botankosho' in 2020.

Varieties	Water stress	Plant Height (cm)	No. of Branches	No. of Leaves	No. of Fruits	Fruit Weight (g)	Total Yield (g)	Brix %
Shishito	E	78.3±1.2 a	13.5±0.4 a	249.0±7.0 a	75.0±0.3 a	10.5±0.1 a	788±33.1 a	5.56±0.06 a
	S	68.0±1.4 a	9.5±0.2 b	160.5±4.0 b	57.5±0.6 b	9.4±0.2 a	518±21.9 b	7.50±0.10 b
	D	44.8±2.2 b	6.8±0.3 c	86.8±2.0 c	21.3±0.7 c	5.4±0.1 b	106± 8.8 c	10.36±0.05 c
Sapporo	E	84.5±2.7 a	11.8±0.5 a	214.0±9.6 a	59.8±2.6 a	8.6±0.1 a	532±23.1 a	6.83±0.55 a
	S	49.0±1.6 b	8.0±0.2 b	166.8±7.9 ab	33.5±1.6 b	8.5±0.1 a	291±14.3 b	7.96±0.06 b
	D	44.3±1.5 b	7.0±0.2 b	114.8±2.8 b	23.8±0.7 b	7.1±0.1 b	169± 4.7 b	10.7±0.02 c
Botankosho	E	85.0±1.7 a	11.8±0.3 a	189.3±9.0 a	29.3±1.4 a	51.0±0.4 a	1404±19.4 a	5.56±0.06 a
	S	62.3±2.0 b	9.3±0.3 a	131.5±2.2 b	24.8±1.0 b	35.8±0.4 b	866± 8.7 b	7.20±0.17 b
	D	47.8±1.4 c	47.8±0.2 b	78.0±4.3 c	13.8±1.9 c	28.5±0.8 c	385± 7.0 c	8.50±0.12 c

E, S and D denotes excess water supply, standard water supply and drought stress, respectively. Treatments with the same letter do not differ significantly within a variety by Tukey's pairwise test ($P < 0.05$). Values are means ± SE. n = 3

Table 6. Effect of salinity stress for plant growth parameters, pod parameters yield per plant and Brix in 'Shishito' and 'Sapporo Oonaga Nanban' in 2020.

Varieties	Salinity Stress	Plant Height (cm)	No. of Branches	No. of Leaves	No. of Fruits	Fruits weight (g)	Total Yield (g)	Brix %
Shishito	E	55.3±1.2 a	5.5±0.14 a	69.5± 6.4 a	42.5±1.1 a	5.2±0.1 a	213± 5.3 a	9.86±0.06 a
	A	66.5±1.0 b	9.0±0.20 b	131.3± 4.8 b	52.8±0.9 b	7.3±0.1 b	369± 6.3 b	8.63±0.05 b
	C	81.0±1.5 c	10.5±0.32 b	209.3± 3.0 c	73.0±1.3 c	9.0±0.1 c	657±11.5 c	7.86±0.01 c
Sapporo	E	34.3±0.7 a	7.3±0.24 a	88.8± 2.0 a	24.3±2.2 a	6.2±0.1 a	150±13.4 a	10.36±0.12 a
	A	43.5±2.1 a	8.0±0.20 a	127.0± 2.2 a	51.5±1.5 b	7.2±0.1 b	371±10.4 b	9.60±0.06 b
	C	62.3±2.9 b	11.0±0.20 b	183.8±12.6 b	69.8±2.9 c	9.2±0.1 c	638±26.4 c	8.73±0.15 c

E, A and C denotes excess salinity stress, additional salinity stress and control, respectively. Treatments with the same letter do not differ significantly within a variety by Tukey's pairwise test ($P < 0.05$). Values are means ± SE. n = 3

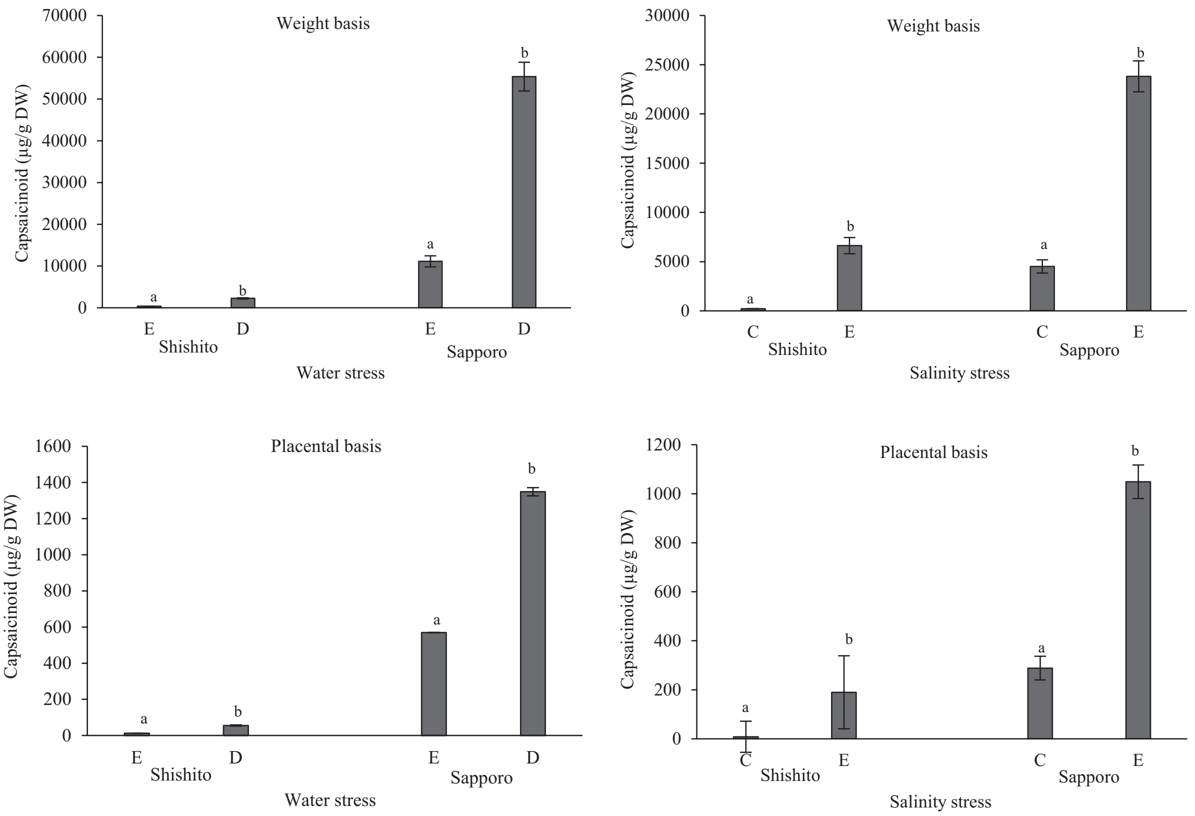

Fig. 2. Capsaicinoids in placental septum of chili peppers on the dry weight basis of fruit and placental septum basis in 'Shishito' and 'Sapporo Oonaga Nanban' for both stress conditions in 2020. In water stress; E and D denotes excess water supply, and drought stress, respectively. In salinity stress; C and E denotes control, and excessive salinity stress, respectively. Treatments with a different letter for the same variety differ significantly by Tukey's pairwise test ($P < 0.05$).

Based on the results in experiment 1 showing that Brix had no correlation with sugar content, we conclude that it is appropriate to use other methods like RQflex than Brix to evaluate fruit sweetness.

Experiment 2 showed a significant positive correlation between total sugar content and Brix in 'Manganji', 'Fushimiamanaga', and 'Botankosho' as individual cultivars. This result indicates that there is a relationship between Brix and total sugar when analyzing individual cultivar rather than when analyzing cultivars as a group. Generally, Brix is an indicator of the total soluble solid in a fruit. In the case of tomatoes, water is the major component, while the remaining portion is solid[4)], which is common with most fruits of any type of species, including chili peppers. Since the moisture content in fruit can differ among varieties, it can affect the concentration of the TSS. Most fruit solids are composed of sugars, organic acids, proteins, minerals, lipids, amino acids, vitamins, pigments, and structural carbohydrates[4, 18)], with soluble sugars and organic acids contributing two-thirds of total solids[3, 6)]. These soluble sugars account for approximately 99% of total fruit sugar content[11, 8)]. Considering this, total sugar content can be a significant factor determining Brix as an indicator of fruit quality. Therefore, an approximation of total sugar content of a fruit can be obtained using Brix.

Finally, when considering data in experiments 1 and 2, measuring Brix in a cultivar group does not give an appropriate estimate of total sugar content. However, measuring Brix for each individual cultivar gives a useful indicator of total sugar content, since it is the major component of the soluble sugars in the fruit and contributes two-thirds of the total fruit solids. Accordingly, when measuring Brix at the farm level, farmers can obtain an estimate of the total sugar content of fruits, but it is valid when measurement is done for an individual cultivar. Measuring Brix is easy and more cost effective than measuring total sugars using RQflex or HPLC methods on site. However, according to Barrett et al[2], Brix may differ among growers, because fruit quality is influenced by grower-specific, including microclimate, soil type, soil nutrients, irrigation methods, moisture-holding capacity of the soil, number of years in cultivation, cultivars, and other unknown factors. Our previous findings for water stress[16] confirmed that drought stress can significantly increase Brix and other taste components of chili pepper fruits, even when other environmental conditions were the same. This finding was reaffirmed for salinity stress conditions, indicating that salinity stress can significantly increase Brix and other taste components of chili pepper fruits[17]. In addition, we found significant increases in capsaicinoid contents in chili peppers caused by stress conditions.

In experiment 1, we found a significant positive correlation between Brix and capsaicinoid content in 'Pungent chili pepper/Chili pepper' group, but in experiment 2, when 'Botankosho' was analyzed, there was no significant relationship between Brix and capsaicinoid content. These results may be simply due to much larger mean Brix of cultivars classified as 'Pungent chili pepper/Chili pepper' group than that of other groups, while capsaicinoids was only recorded in the 'Pungent chili pepper/Chili pepper' group. Due to the smaller size and lower moisture content of fruit than other two groups, mean Brix was larger, and this may be the reason for the apparent correlation with capsaicinoid content. Thus, there may not be any relationship between the two taste components, and in experiment 2, we found that 'Botankosho' did not have any correlation.

In experiment 3, when plants were grown under drought or excessive salinity stress conditions, Brix became larger than when grown under excess water or normal conditions. This tendency was common in almost all varieties and treatments in both experiments. Rathnayaka et al.[16] found that Brix significantly increased in chili pepper fruits when plants are under drought stress. The present results agreed with this for all cultivars under drought stress. Rathnayaka et al.[17] also found that Brix significantly increased in chili pepper fruits when plants are under salinity stress condition. The results in experiment 3 obtained where excess salinity stress conditions were given also agreed with those of Rathnayaka et al.[17].

Similarly, stress also reduced fruit yield and fruit size. Therefore, the increase of Brix in the fruits was inferred to be due to the concentration of sugar resulting from reduced number and size of fruits. However, stress also reduced the number of leaves, which should have limited the supply of sugar. The present results suggest that the sugar content in fruits increased because the sugar accumulation due to the decrease in fruit number and size was greater than the decrease in photosynthetic capacity caused by the stress-induced decrease in leaf number.

Capsaicinoid content showed a similar response to stress conditions and Brix. The highest content of capsaicinoids in placental septum was observed in the drought and excess salinity stress treatments. According to Estrada et al.[5], stronger pungent chili fruits are found when chili peppers are grown under drought stress. Moreover, Rafael et al.[15] revealed that capsaicin and dihydrocapsaicin levels were the highest in high salinity treatments. We found that the capsaicinoid content per weight (μg/g DW) of the placental septum was significantly increased by stress.

The size of fruits in drought and excess salinity stress conditions was small, while that in excess water and control salinity conditions was comparatively larger. If the placental septum size was not changed by the stress conditions even though the fruit size was reduced, and if the capsaicinoid synthesis capacity per fruit/placental septum will not change, then it may be that capsaicinoids was concentrated because of the small fruit size. Therefore, we analyzed the data in regard to stress conditions and capsaicinoids in the placental septum not on a weight basis but on a per placental septum basis (μg/fruit). However, in the present experiment, we found that the placental septum size was also reduced by stress condition (Fig. 3). According to these two analyses, it seems that the higher amount of capsaicinoids in stress conditions was not due to the concentration of them in a smaller fruit area (Fig. 2) but to accelerated capsaicinoid synthesis

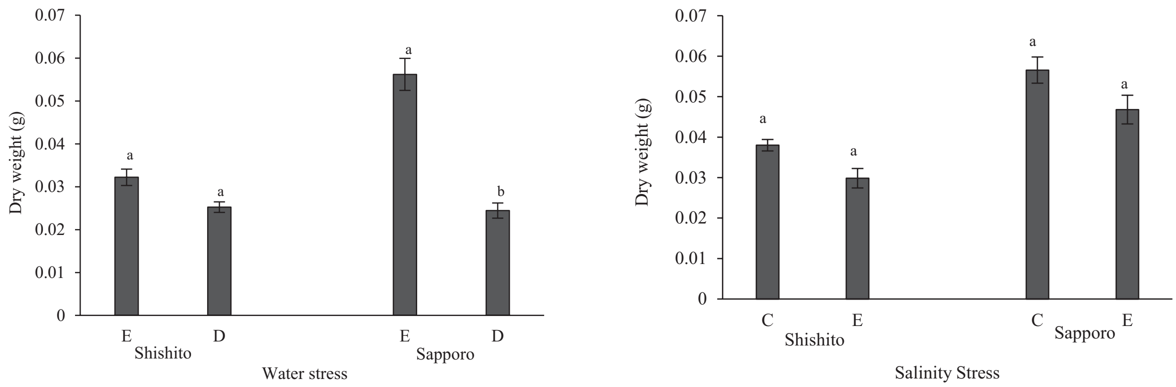

Fig. 3. Dry weights of placental septum in chili peppers 'Shishito' and 'Sapporo Oonaga Nanban' in 2020. In water stress; E and D denotes excess water supply, and drought stress, respectively. In salinity stress; C and E denotes control, and excessive salinity stress, respectively. Treatments with the same letter do not differ significantly within a variety by Tukey's pairwise test ($P < 0.05$).

caused by stress conditions.

The present study was conducted to clarify the relationship between fruit Brix and other chemical components related to the taste of chili peppers and to clarify how Brix, which changes in response to environmental stress, is related to other traits such as yield. Our results clarified that it is not appropriate to use Brix to compare varieties in terms of total sugar content, but it is possible to estimate the differences among treatments and individuals within the same variety. However, it is better to perform spectrophotometry or HPLC analyses in a laboratory to obtain exact information about sugar content.

We also found that stress during cultivation increased Brix and capsaicinoid content, but also decreased yield, fruit size, and number of leaves. The stress-induced reduction in Brix could be due to the reduction in yield and fruit size, but similarly, the negative impact of reduced photosynthetic capacity due to reduced number of leaves on Brix was inferred to be less. However, the stress-induced increase in capsaicinoids content was not due to a decrease in fruit size, but rather due to an enhanced ability to synthesize capsaicinoids in the placental septum.

In the future, it will be necessary to elucidate the genetic mechanisms by conducting the expression analyses of the genes involved in the synthesis and accumulation of sugars and capsaicinoids when plants are under stress conditions.

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トウガラシにおける Brix と諸形質との関係

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本研究は，トウガラシ果実の Brix と各種呈味成分との関係を明らかにするとともに，環境ストレスに応じて変化する Brix と収量等諸形質の関係を明らかにすることを目的とした．研究は2015年から2019年にかけて実施した．実験1では，2015年に39品種の *Capsicum annuum* を栽培し，辛味および呈味成分を評価した．また，品種間の辛味および呈味成分の関係についても調査した．実験2では，2015年から2019年にかけて，日本産在来トウガラシ品種‘万願寺’，‘伏見甘長’，‘ぼたんこしょう’をストレス条件下で栽培し，個々の品種における辛味および呈味成分の関係を調べた．実験3では，‘ししとう’，‘札幌大長’，‘ぼたんこしょう’の各品種について，乾燥ストレスと塩分ストレスを用いて，ストレス条件が植物の生育，収量，Brix および辛味成分含量に与える影響を調査した．実験1では，ピーマン・パプリカ品種群，甘味トウガラシ品種群，辛味トウガラシ品種群，および全ての品種・系統を用いた場合のいずれも総糖度とグルコース含量との間に有意な正の相関が認められたが，Brix と総糖度との間には有意な正の相関は認められなかった．一方で，実験2では，品種ごとにみると総糖度と Brix との間に有意な正の相関が認められた．これらの結果から，Brix を用いて総糖度の品種間差を比較することは適切ではないが，同一品種であれば，処理間や個体間の総糖度を Brix で推定することは可能であることが明らかになった．また，実験3では，栽培中のストレスは Brix とカプサイシノイド含量を増加させるが，収量，果実サイズ，葉数を減少させることが明らかになった．ストレスが果実の Brix を増加させるのは，果実収量や果実サイズを減少させることによるもので，葉数の減少を通じて光合成能力を低下させることによる Brix への影響は小さいと推察された．

キーワード：Brix，カプサイシノイド，*Capsicum annuum*，可溶性固形分，総糖度