

# No Effect of Seed Presence or Absence on Sugar Content and Water Status of Seeded and Seedless Watermelon Fruits

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**Abstract.** We investigated physiological differences in watermelon [*Citrullus lanatus* (Thunb.) Matsum. et Nakai] fruits among seeded diploid and seedless triploid fruits, N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU)-treated seedless fruits, and soft-X-irradiated pollen-pollinated seedless fruits to investigate the effect of the presence or absence of seeds on water relations and sugar content. We picked fruits at 20 and 40 days after anthesis and sampled flesh at the center, around the seeds, and near the pericarp to measure water status and sugar content. There were no significant differences between seeded and seedless cultivars in sugar contents or in water and osmotic potentials of the flesh, although the latter two were decreased at 40 days. CPPU and soft-X-irradiated pollen eliminated mature seeds, but there were again no significant differences in sugar contents or water status between seeded and seedless fruits. Thus, the presence or absence of seeds did not influence the sugar content or osmotic pressure in watermelon fruit, so sugar accumulation was not related to seeds.

Consumers favor seedless fruits for eating in both fresh (e.g., grape, citrus, and banana) and processed forms (e.g., tomato sauce). Seedlessness can improve fruit quality by consumer preference (Pandolfini, 2009). Marr and Gast (1991) reported that consumers were willing to pay 50% more for seedless watermelons. To produce seedless watermelons, techniques such as triploidy, hormonal treatment, and pollination with inactivated pollen have been tried. Seedlessness may affect fruit quality. Triploid cultivars tended to have higher soluble solid contents (Brix) than diploids (Maynard et al., 2002; Pardo et al., 1997). Seedless fruits induced by  $\gamma$ -irradiated pollen had significantly higher sugar content than seeded fruits (Moussa and Salem, 2010). On the other hand, Leskovar et al. (2004) found that total sugar content in fruit was not significantly different among diploid and triploid cultivars. Hayata et al. (1995) found no consistent relationship between sugar content and the application of CPPU. Furthermore, the sugar content of seedless fruits induced by soft-X-irradiated pollen was similar to or higher than that of controls (Sugiyama and Morishita, 2000). Thus, the role of seeds in sugar accumulation in watermelon is still uncertain.

To investigate the effect of the presence or absence of seeds on sugar content in watermelon fruits, we designed 3 experiments: Expt. 1, comparing diploid seeded 'Hitorijime-BonBon' and triploid seedless 'Sandia' fruits; Expt. 2, testing the effect of CPPU; and Expt. 3, pollinating flowers with soft-X-irradiated pollen.

We also examined hydrostatic pressure, which is generated by solutes in solution (in this case, cell sap). Seeds act as an assimilate sink during fruit growth. Most sinks in higher plants import assimilates by bulk flow driven by differences in hydrostatic pressure (Patrick et al., 2001). Gradients in water potential in the transport pathway have been measured in wheat grains (Fisher and Oparka, 1996), strawberry (Pomper and Breen, 1995), and grape berries (Lang and Doring, 1991). Molecules that are known to control osmotic pressure include sugars, glycerol, amino acids, sugar alcohols, and various low-molecular-weight metabolites (Boyer, 1995b). Thus, we also measured water status.

We analyzed sugar contents by high-performance liquid chromatography (HPLC), and water status [water potential, osmotic potential ( $\psi_s$ ) = osmotic pressure, turgor pressure] with an isopiestic thermocouple psychrometer, and measured seed size during fruit growth.

## Materials and Methods

*Plant materials.* We grew two cultivars of watermelon [*C. lanatus* (Thunb.) Matsum. et

Nakai]: the seeded diploid 'Hitorijime-BonBon' and the seedless triploid 'Sandia' (Hagihara Farm Co., Ltd., Nara, Japan). Both cultivars are suitable for harvesting by 40 d after pollination. We obtained seedlings in May 2015–17 and transplanted them into three hydroponic culture beds (258 cm  $\times$  110 cm  $\times$  15 cm) at 50 cm between plants (5 plants  $\times$  2 lines were grown in each bed). Nutrient solution (Otsuka Chemical Co., Ltd., Osaka, Japan) with an electric conductivity (EC) of 1.2 mS $\cdot$ cm<sup>-1</sup> and a pH of 5.8 was maintained at a depth of 10 cm using EC meter (Atago Co., Ltd., Tokyo, Japan) and pH meter (Thermo Fisher Scientific K.K., Kanagawa, Japan). The beds were maintained in a glasshouse that was ventilated whenever the air temperature exceeded 25 °C.

In 2015 (Expt. 1), female flowers of each cultivar were pollinated conventionally by hand. In 2016 (Expt. 2), half of the female flowers of 'Hitorijime-BonBon' were pollinated conventionally and the other half were sprayed with 0.5 mL of CPPU solution ( $2 \times 10^{-3}\%$ ) (Kyowa Hakko Kogyo Co., Tokyo, Japan) per flower to induce seedless fruits. In 2017 (Expt. 3), half of the female flowers of 'Hitorijime-BonBon' were pollinated conventionally and the other half were hand-pollinated with soft-X-irradiated pollen (OREC Co., Fukuoka, Japan).

The plants were grown vertically (Watanabe et al., 2001). Three vines per plant were trained upward and all other vines were removed. When each vine had produced more than 20 nodes, one fruit was retained on it (three fruits per plant). The fruits were supported on a ball net (Molten Co., Hiroshima, Japan) on bars placed at 230 cm height. We measured the fruit circumferences every 10 d with a measuring tape. We collected fruits at 20 and 40 d after pollination or CPPU treatment, and sampled 2-cm cubes from similar positions in the center, around the seeds, and near the pericarp for measurement of both water status and sugar contents. In Expts. 2 and 3, we also counted the mature and immature seeds in two or three fruits.

*Sugar contents.* We analyzed the sugar contents and composition of the flesh by HPLC (EZChrom Elite; Hitachi High-Technologies Co., Tokyo, Japan). Squeezed juice (1 mL) was centrifuged (18,845 g) for 2 min at 10 °C, diluted with water (juice: water = 1:2–5), and filtered through a 0.45- $\mu$ m HPLC filter (GE Healthcare Life Sciences Co., Buckinghamshire, UK). Sugars were separated in an analytical HPLC system fitted with a 5- $\mu$ m TSKgel Amide-80 column (Tosoh Co., Tokyo, Japan) kept at 80 °C. The mobile phase was acetonitrile and distilled water (80:20), and the flow rate was 1.0 mL $\cdot$ min<sup>-1</sup>. All data differences were analyzed at 5% significant level by Student's *t* test.

*Water status.* We measured the water status of the flesh with an isopiestic psychrometer (Model-3; Isopiestic Psychrometry Ltd., Lewes, DE) (Boyer, 1995a). The thermocouple chambers were coated with petrolatum and were then loaded with tissue samples immediately after sampling. After the water potential was measured, the  $\psi_s$  was measured

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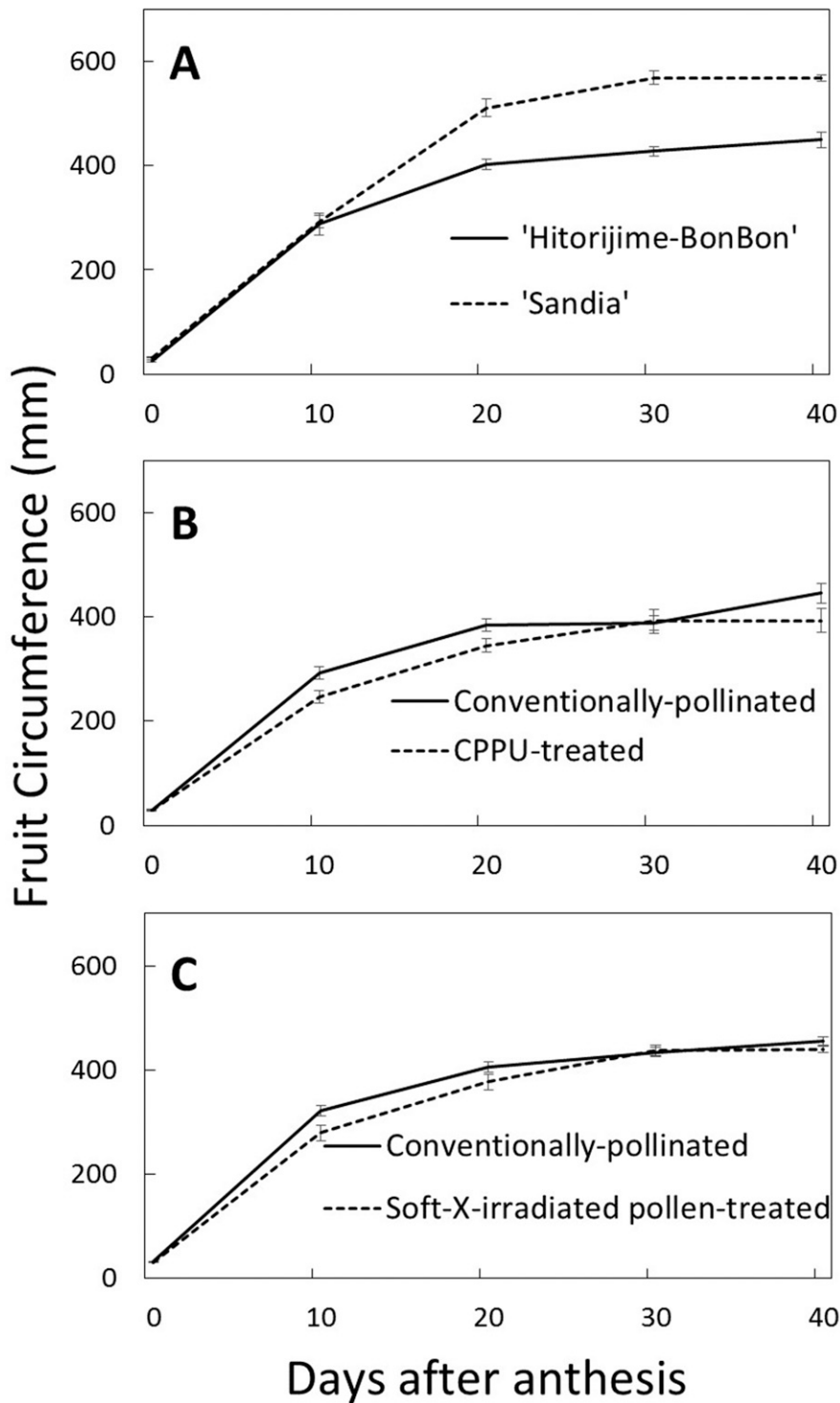


Fig. 1. Fruit circumferences of (A) diploid 'Hitorijime-BonBon' and triploid 'Sandia' (Expt. 1), (B) conventionally pollinated and CPPU-treated 'Hitorijime-BonBon' (Expt. 2), and (C) conventionally pollinated and soft-X-irradiated pollen-pollinated 'Hitorijime-BonBon' (Expt. 3) during growth. Error bars represent SE ( $n = 4-10$ ). CPPU = N-(2-chloro-4-pyridyl)-N'-phenylurea.

after freezing at  $-80\text{ }^{\circ}\text{C}$  and then thawing (Boyer, 1995a). Turgor pressure was calculated as the water potential minus the  $\psi_s$ . All data differences were analyzed at 5% significant level by Student's  $t$  test.

## Results

**Fruit growth and seed profiles.** The circumferences of all fruits increased quickly

until 20 d after pollination and then gradually until 40 d, reaching 550 mm in 'Sandia' (Fig. 1A) and 400 mm in all seeded and seedless 'Hitorijime-BonBon' fruits (Fig. 1). Both CPPU treatment and pollination with soft-X-irradiated pollen eliminated mature seeds (Table 1).

**Expt. 1: Triploid versus diploid fruits** (Figs. 2 and 3). The total sugar content was low in all three locations at 20 d after

Table 1. Seed profiles in conventionally pollinated, CPPU-treated, and soft-X-irradiated pollen-pollinated 'Hitorijime-BonBon' fruits harvested at 40 d.

	Number of seeds	
	Mature	Immature
Expt. 2		
Conventionally pollinated	176	66
CPPU-treated	0	240
Expt. 3		
Conventionally pollinated	238	25
Soft-X-irradiated pollen-treated	0	234

Data are means of two or three fruits.

CPPU = N-(2-chloro-4-pyridyl)-N'-phenylurea.

pollination (Fig. 2D) but more than doubled at 40 d in the center and around the seeds (Fig. 2H) in both cultivars. The water potential was always least negative near the pericarp and all values became lower at 40 d than at 20 d in both cultivars (Fig. 3A and D). Osmotic potential showed a similar tendency (Fig. 3B and E). Although turgor pressure was similar at all positions at 20 d (Fig. 3C), it became very low in the center and around the seeds at 40 d (Fig. 3F).

**Expt. 2: CPPU treatment** (Figs. 4 and 5).

Total sugar contents were low at 20 d (Fig. 4D) but doubled at 40 d in the center and around the seeds in both treatments (Fig. 4H). The water potential was similar at all positions at 20 d (Fig. 5A) and then decreased at 40 d, especially around the seeds and in the center (Fig. 5D). Osmotic potential showed a similar tendency (Fig. 5B and E). Neither differed significantly between treatments, even at 40 d. Turgor pressure was  $>0.2$  MPa at all sample positions at 20 d (Fig. 5C), but decreased to near 0 MPa in the center and around the seeds at 40 d (Fig. 5F).

**Expt. 3: Soft-X-irradiated pollen** (Figs. 6 and 7). Total sugar contents were low at 20 d (Fig. 6D) but more than doubled at 40 d in the center and around the seeds in both treatments (Fig. 6H). The water potential was similar at all positions at 20 d (Fig. 7A), when we compared the treated and nontreated fruits, and then became lower at 40 d (Fig. 7D). Osmotic potential showed a similar tendency (Fig. 7B and E). Neither differed significantly between treatments, even at 40 d. Turgor pressure was  $>0.2$  MPa at all sample positions at 20 d (Fig. 7C), but decreased to near 0 MPa in the center and around the seeds at 40 d (Fig. 7F).

## Discussion

In all experiments, triploid 'Sandia', CPPU-treated diploid 'Hitorijime-BonBon', and soft-X-irradiated pollen-pollinated 'Hitorijime-BonBon' produced no mature seeds (Table 1, data shown for Expts. 2 and 3). Similarly, watermelon fruits induced by CPPU or by 800-Gy soft-X-irradiated pollen produced no mature (hard black) seeds (Hayata et al., 1995; Kwon et al., 2006; Sugiyama and Morishita, 2000). We regarded small

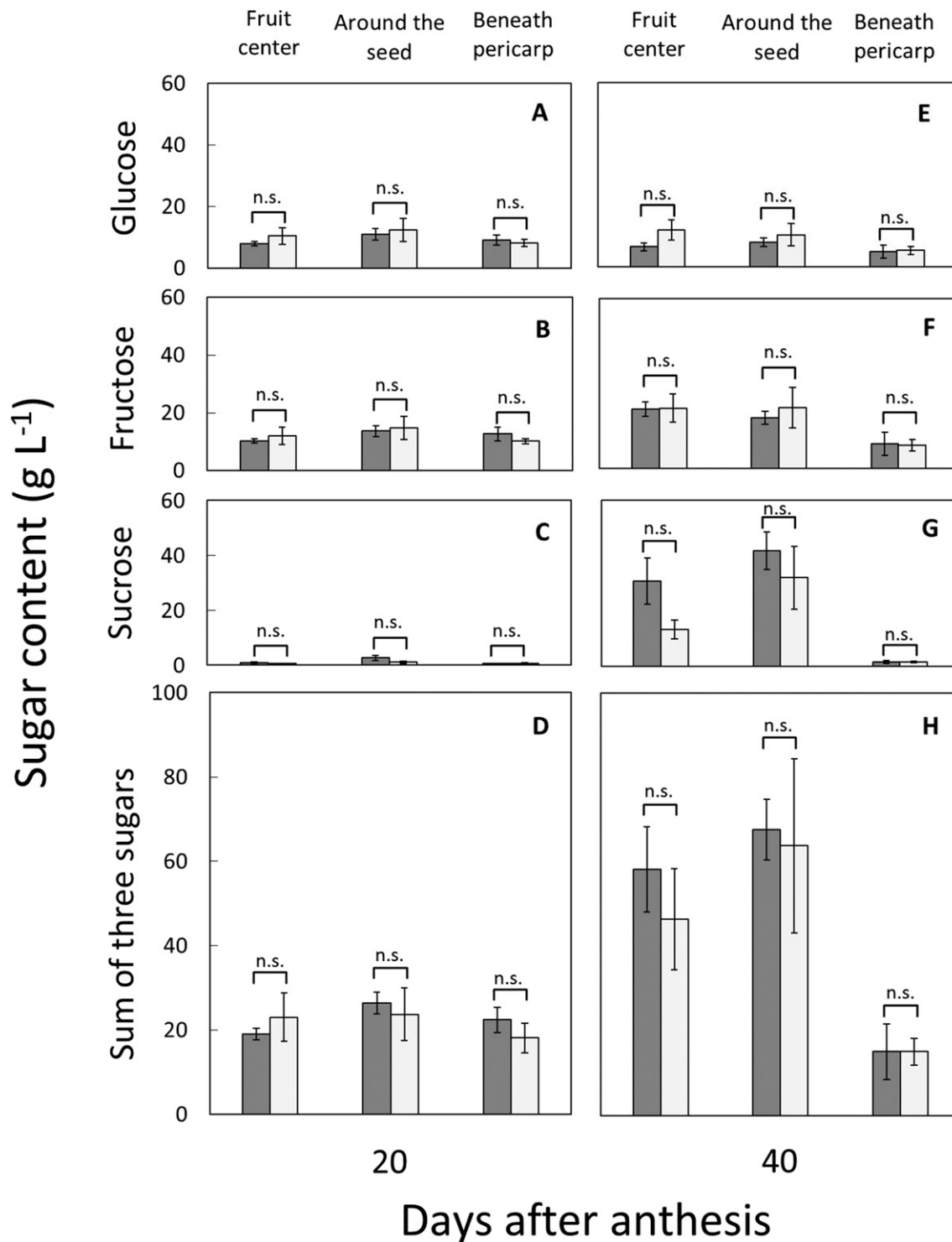


Fig. 2. Contents of (A and E) glucose, (B and F) fructose, (C and G) sucrose, and (D and H) total sugars in ■ 'Hitorijime-BonBon' and □ 'Sandia' fruits at (A–D) 20 and (E–H) 40 d after anthesis. Error bars represent SE. <sup>n.s.</sup>Not significantly different between cultivars ( $n = 3-5$ ) at  $P > 0.05$  by Student's  $t$  test.

white seeds as immature remnants of limited ovule development (Hayata et al., 1995). Fruits with such aborted white seeds, regarded as seedless, were similar in size to normally pollinated seeded fruits (Kwon et al., 2006; Sugiyama and Morishita, 2000). Our results support the idea that seedlessness is not related to fruit size. Although Hayata et al. (1995) reported that

20-ppm CPPU induced smaller fruits, we found that it produced similar-size fruits to conventional pollination (Fig. 1B). The reason for the difference is unclear, but culture conditions (e.g., hydroponic; vertical) might affect fruit size. However, 200-ppm CPPU (Hayata et al., 1995) and 50-ppm CPPU (Kwon et al., 2006) induced fruits of a similar size to normally pollinated fruits.

Thus, CPPU appears not to reduce fruit size in 'Hitorijime-BonBon'.

There were no differences between cultivars or treatments in sugar content or water status (Figs. 2–7) except in water status at 40 d in Expt. 1, in which 'Sandia' had lower water and  $\psi_s$  than 'Hitorijime-BonBon' (Fig. 3D and E). As sugar content was unaffected in either cultivar, other mineral or

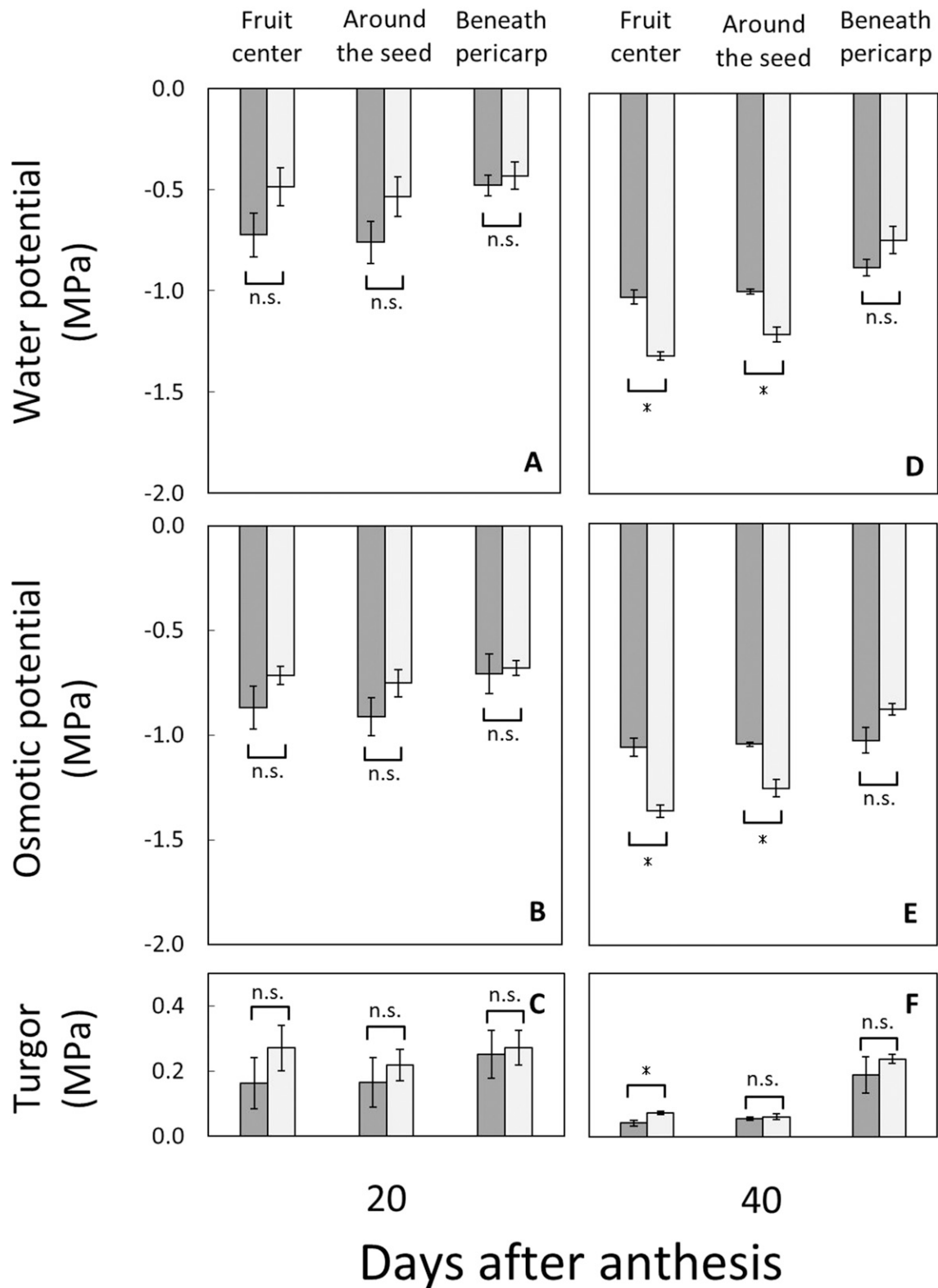


Fig. 3. (A and D) Water potential, (B and E) osmotic potential, and (C and F) turgor pressure of ■ 'Hitorijime-BonBon' and □ 'Sandia' fruits at (A–C) 20 and (D–F) 40 d after anthesis. Error bars represent SE. \*Significantly different between cultivars ( $n = 3-5$ ) at  $P < 0.05$  by Student's  $t$  test.

organic solutes accumulated in the fruits of 'Sandia'. These results were similar to those obtained in triploid cultivar SL-S2 (Kawamura et al., 2016).

In other plant species also, the presence of seeds was not related to sugar accumulation in fruits. Zhang et al. (2008) reported that

Brix in pear was unaffected by CPPU induction of seedlessness. Bangerth and Schroder (1994) similarly reported that the Brix in apple was unaffected by GA (gibberellin) + CPPU induction of seedlessness. In contrast, Fujishima et al. (2012) reported that seeded 'Kyoho' grapes tended to have a higher Brix

than GA-treated seedless grapes. Weinbaum et al. (2001) reported that seeded pear fruits averaged 12% heavier than facultative seedless pears. As described in the Introduction, conflicting reports about the relationship between sugar accumulation and the presence or absence of seeds in watermelon

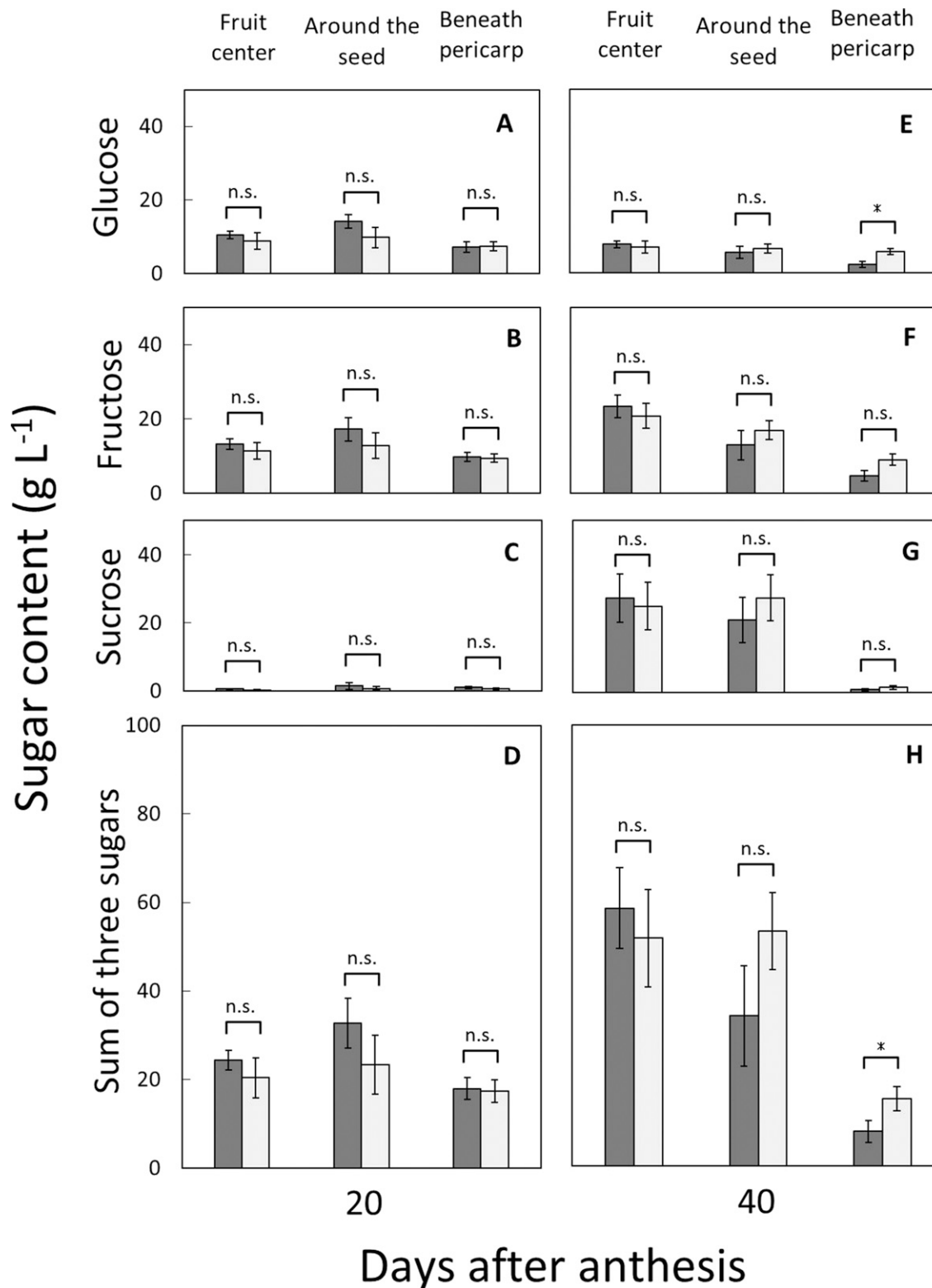


Fig. 4. Contents of (A and E) glucose, (B and F) fructose, (C and G) sucrose, and (D and H) total sugars in ■ conventionally pollinated and □ CPPU-treated 'Hitorijime-BonBon' fruits at (A–C) 20 and (G–I) 40 d after anthesis. Error bars represent SE. \*Significantly different between treatments ( $n = 4-6$ ) at  $P < 0.05$  by Student's  $t$  test. CPPU = N-(2-chloro-4-pyridyl)-N'-phenylurea.

fruits obscure any relationship. Our three methods of inducing seedlessness indicated that there is no relationship between sugar accumulation and seed existence, as in earlier works inducing seedless watermelons with CPPU (Hayata et al., 1995)

and soft-X-irradiated pollen (Sugiyama and Morishita, 2000).

Most earlier studies used soil culture. Because it can be difficult to control soil water status, we used hydroponic culture to maintain plant water status. A water

potential gradient is required to move ions and sugars into fruits (Boyer, 1995a). Osmotic pressure is controlled by sugars, glycerol, amino acids, sugar alcohols, and various low-molecular-weight metabolites (Boyer, 1995b). Both water potential and

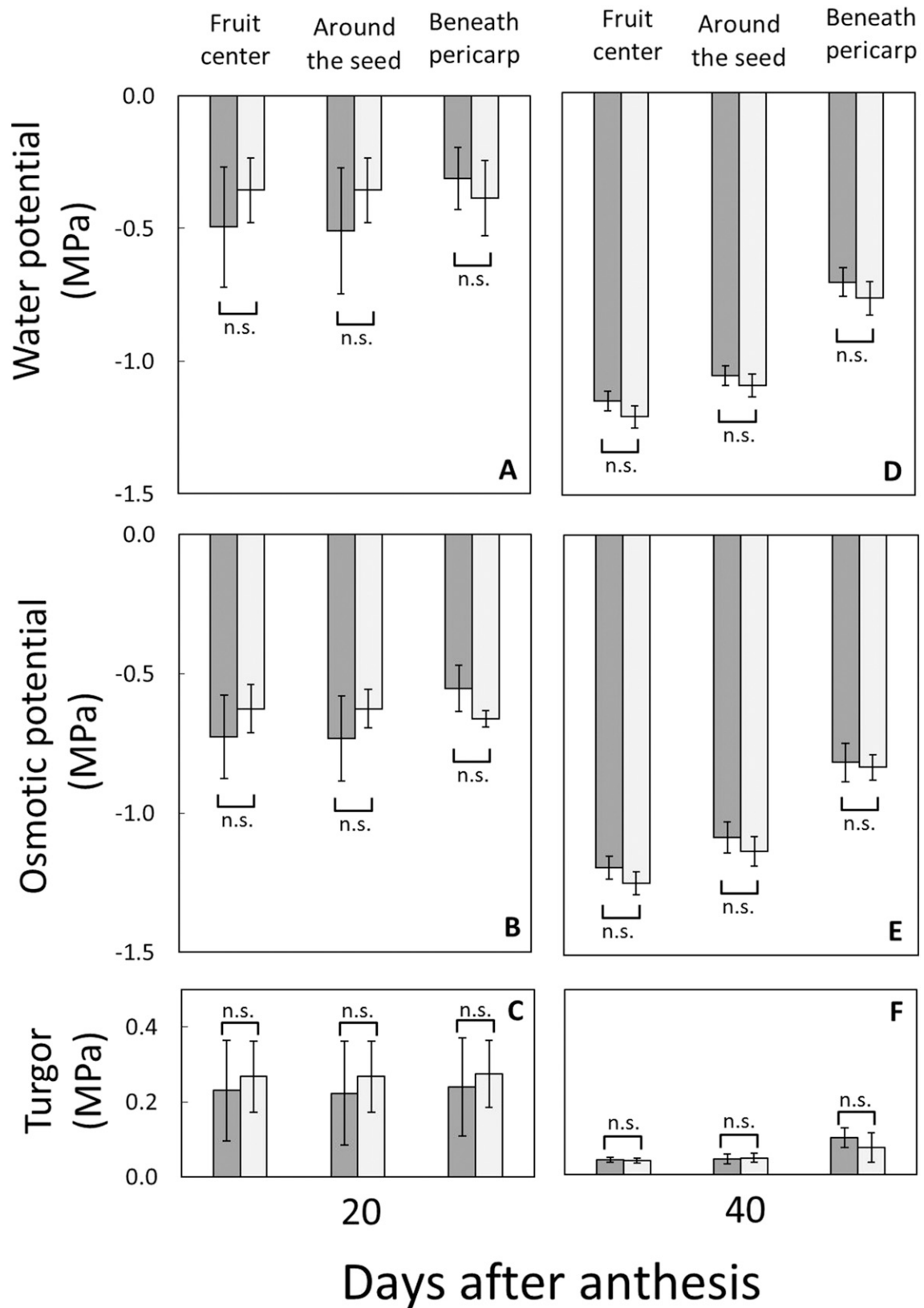


Fig. 5. (A and D) Water potential, (B and E) osmotic potential, and (C and F) turgor pressure of ■ conventionally pollinated and □ CPPU-treated ‘Hitorijime-BonBon’ fruits at (A–C) 20 and (D–F) 40 d after anthesis. Error bars represent SE. <sup>n.s.</sup>Not significantly different between treatments ( $n = 4-6$ ) at  $P > 0.05$  by Student’s  $t$  test. CPPU = N-(2-chloro-4-pyridyl)-N’-phenylurea.

$\psi_S$  became lower at the center of the fruits and around the seeds from 20 to 40 d after anthesis (Figs. 3, 5, and 7). This lower  $\psi_S$  means that sugars accumulated at both sites

in watermelon fruits (Figs. 2, 4, and 6). Furthermore, sugar content more than doubled and  $\psi_S$  decreased greatly at 40 d, suggesting that sugar contributes to the

decreased  $\psi_S$  when fruits mature. During the growth of ‘Yamatokomachi’ watermelon fruits, sugar accounted for 47% to 66% of the total  $\psi_S$  (Ikeda et al., 2011). Our results show

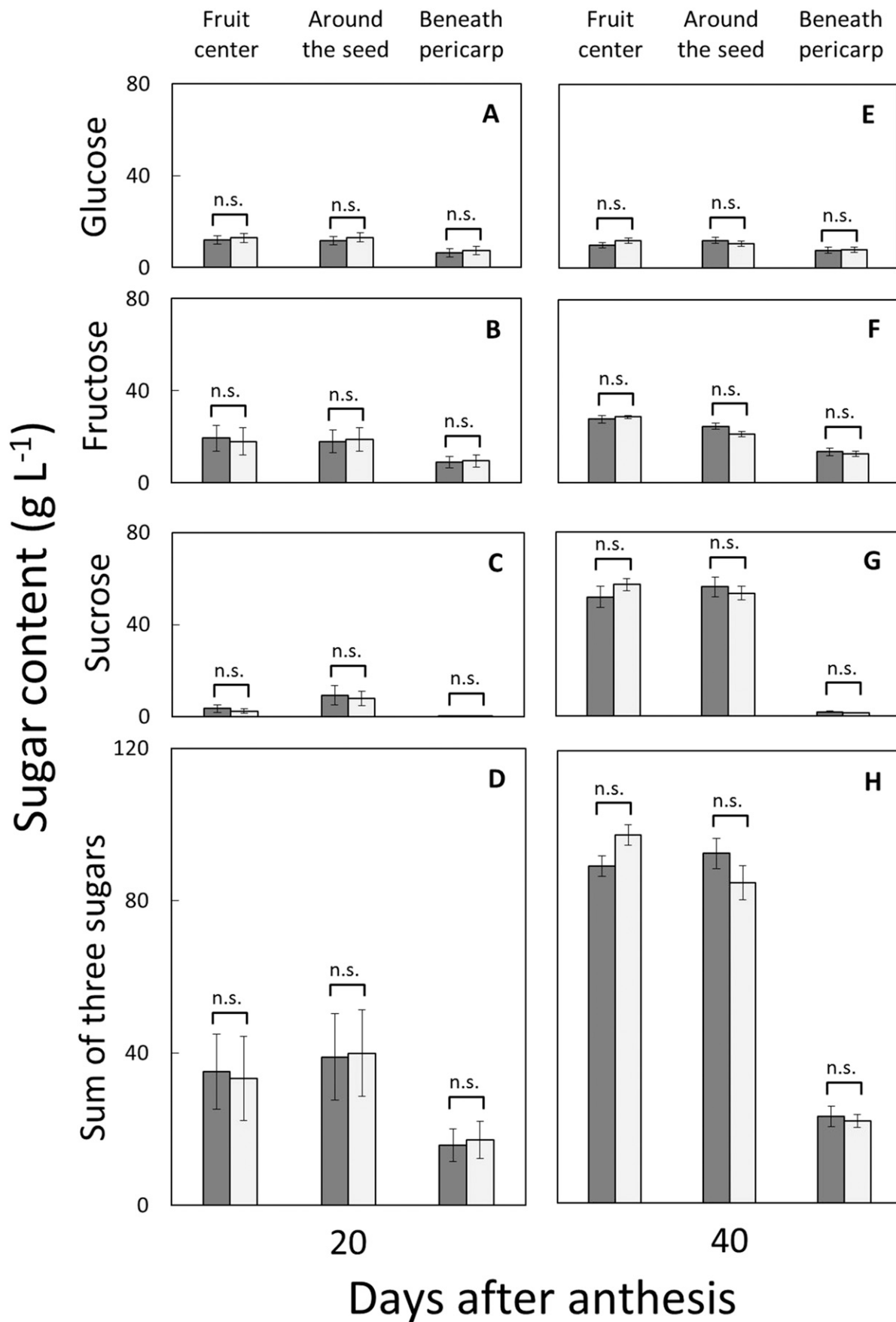


Fig. 6. Contents of (A and E) glucose, (B and F) fructose, (C and G) sucrose, and (D and H) total sugars in ■ conventionally pollinated and □ soft-X-irradiated pollen-pollinated 'Hitorijime-BonBon' fruits at (A–C) 20 and (G–I) 40 d after anthesis. Error bars represent SE. \*Significantly different between treatments ( $n = 5$  or 6) at  $P < 0.05$  by Student's  $t$  test.

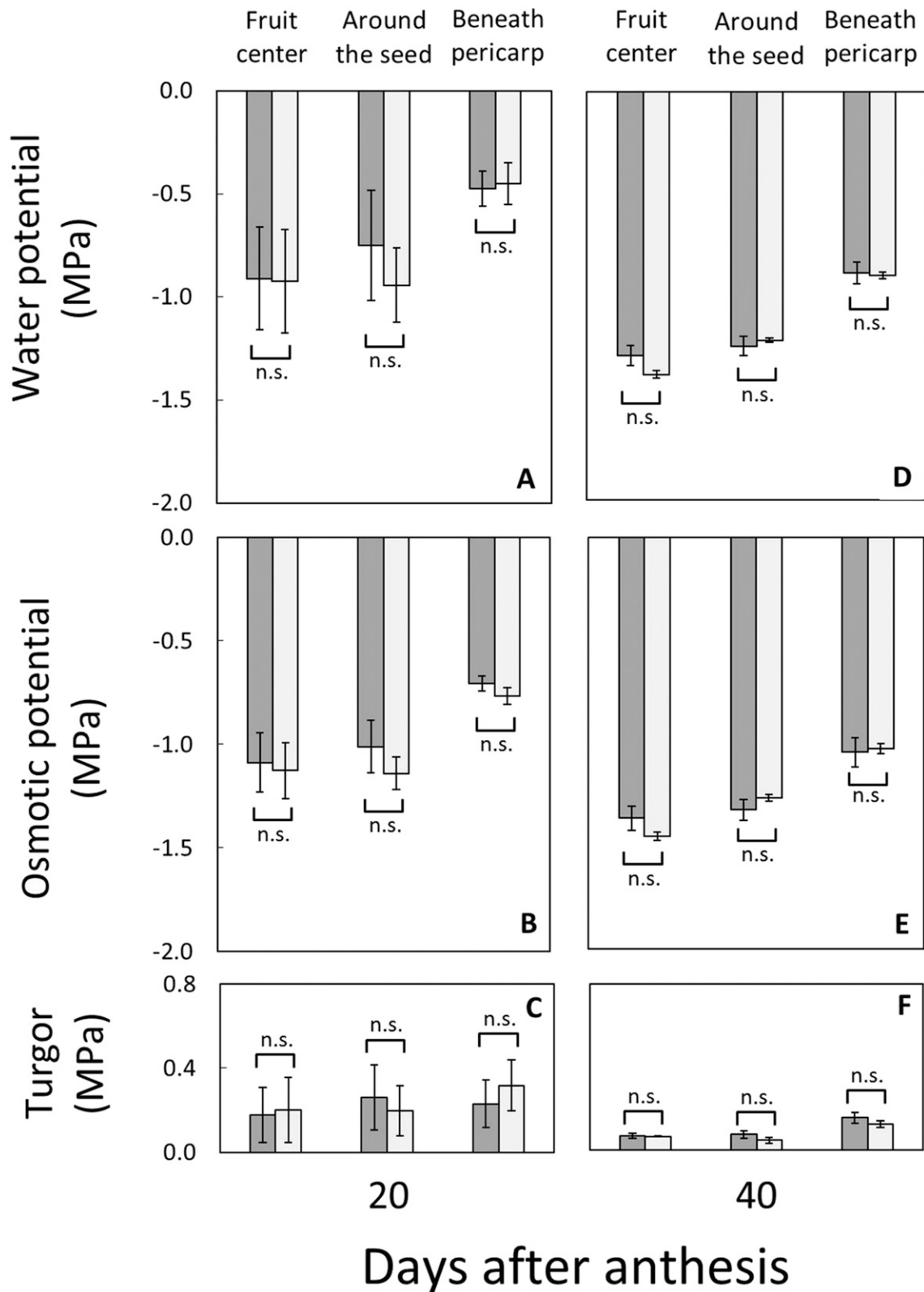


Fig. 7. (A and D) Water potential, (B and E) osmotic potential, and (C and F) turgor pressure of ■ conventionally pollinated and □ soft-X-irradiated pollen-pollinated 'Hitorijime-BonBon' fruits at (A–C) 20 and (D–F) 40 d after anthesis. Error bars represent SE. <sup>n.s.</sup>Not significantly different between treatments ( $n = 5$  or 6) at  $P > 0.05$  by Student's  $t$  test.

that seeds are not related to the osmotic contribution of sugar.

CPPU is a synthetic cytokinin that promotes cell division (Kano, 2000; Okamoto et al., 1981). Cytokinins induce extracellular

invertase, which supplies carbohydrates for growth (Roitsch and Ehneß, 2000). X-irradiation of pollen induces genetic abnormalities. Sugiyama and Morishita (2002) postulated that chromosomal abnormalities

induced with soft X-rays in the generative nucleus result in embryo abortion. Despite the large differences in the mechanisms of the induction of seedlessness, our results were similar between seeded and seedless fruits.



We conclude that there was no difference in sugar content between seeded and seedless watermelons, confirmed by measuring water status and analyzed sugar contents in fruits.

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