Effects of Temperature around the Fruit on Sugar Accumulation in Watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) during the Latter Half of Fruit Developmental Period

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Nursery plants of watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) were planted in the field on April 19, 2006. To examine the correlation between temperatures around fruit during the latter half of the fruit developmental period and sugar accumulation in fruit, fruits were covered with polyethylene (PE) film, infrared-ray-blocking (IRB) film, and black nylon mesh (BNM) from 20 to 48 days after anthesis (DAA). Relative to the untreated control, higher fruit temperatures were detected by PE film-covering, but the temperatures remained low by IRB and/or BNM film-covering. Fruit growth was significantly accelerated by PE film-covering, but IRB and BNM film-covering resulted in a reduction of fruit growth. Cell enlargement at the intermediate and outer regions of fruit progressively intensified in the former as compared with the latter. Higher fructose and sucrose contents were detected in the intermediate and outer regions of fruit by IRB film-covering, while PE film-covering decreased glucose and fructose contents in all regions of fruit. These results suggest that the active cell enlargement caused by higher temperature during the latter half of the fruit developmental period is closely correlated with the reduction of sugar content in fruit, and that higher sugar content can be produced by lowering temperature with IRB film-covering in this period.

Key Words: Cell size, fruit temperature, sugar accumulation, watermelon.

Introduction

Watermelon (Citrullus lanatus (Thunb.) Matsum. & Nakai) is extensively cultivated on the sand dunes of Ishikawa Prefecture, Japan. The shipping of watermelons begins from early June to the end of July. According to growers in Ishikawa Prefecture, the sweetness of watermelons harvested after mid-July is not high enough, resulting in a low value in the market.

A negative correlation between sugar content and exposed air temperature during the latter half of the fruit developmental period has been observed in many fruit crops cultivated in summer. For example, sugar contents in Satsuma mandarin oranges (Utsunomiya et al., 1982) and ‘Delaware’ grapes (Kobayashi et al., 1967) are suppressed under higher air temperature, approximately 30°C. Kano (2004) proposed that high temperature conditions around watermelon fruit during the latter half of the fruit developmental period were one of the main reasons for lower sugar content in midsummer. Seyama and Abe (1977) showed that direct solar radiation raised the fruit temperature of tomato up to 45°C. Likewise, Odagiri et al. (1987) recognized that the fruit surface temperature of watermelon in midsummer often exceeded 30°C. According to Kano (2004), watermelon fruit with high sugar content could be produced by lowering day time temperature by shading only the fruit. These findings suggest that watermelons heated by strong solar radiation in midsummer during the latter half of the fruit developmental period brings about lower sugar content in fruit.

The present investigation was carried out to clarify the effect of temperature conditions around watermelon fruit during the latter half of the fruit developmental period on sugar accumulation, and to evaluate the use of infrared-ray-blocking film around fruits in this period.

Materials and Methods

Plant materials

Watermelon seeds (‘Kansen’) were sown on February 24, 2006, and the resulting seedlings were grafted onto
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Bottle gourd (‘Kachidoki-No. 2’; Lagenaria siceraria Standl. var. hispida Hara) rootstock on March, 28. Sixty g·m⁻² each of dolomite, 8.8 g·m⁻² N, 17.1 g·m⁻² P₂O₅, 8.6 g·m⁻² K₂O, and microelemental manure containing 0.4% Mn, 0.3% B, 1.2% Fe, and 0.03% Cu were applied to the beds approximately one week before transplanting. Nursery plants were spaced at 40 cm intervals in rows 300 cm wide on April 19. The beds were covered with white polyethylene mulch 180 cm wide. A white polyolefin row cover (0.05 mm thick) was installed at planting time, suspended on arches 50 cm high and 180 cm wide to form a tunnel above the rows, until approximately 10 days after anthesis (DAA). Primary shoots were pinched off at the end of April and two lateral shoots were allowed to grow. A side dressing of 3.6 g·m⁻² N, 3.2 g·m⁻² P₂O₅, and 3.6 g·m⁻² K₂O was applied at the end of May. The third flowers, which emerged mostly at the 18th node of each secondary vine, were hand pollinated, and thereafter fruit was removed so that one fruit was left on each stock in mid-June.

To examine the effect of temperature during the latter half of the fruit developmental period on sugar accumulation, tunnels 50 cm high and 50 cm wide were laid above the fruits. These tunnels were covered with polyethylene (PE) film (0.03 mm thick), infrared-ray-blocking (IRB) film (Mega-cool, MKV Platech, Japan; 40–60% transmittance of solar radiation above 700 nm), or black nylon mesh (BNM, Dionet 810, Dio Chemicals, Japan; 60% shading of full solar radiation) from 20 to 48 DAA. PE film was used to raise the temperature around fruit, and IRB and BNM were used to lower temperature. Control plants were grown without any treatment. Five fruits were sampled at each of 30, 40, and 48 DAA, and weighed. Three uniform fruits were used for sugar analysis.

Sugar analysis

Slices approximately 1 cm thick were cut from the maximum transverse diameter toward the peduncle end of the watermelon fruit. As shown in Figure 1, three tissue samples were taken from each slice: one from the central region, one from the outer region, and one from the intermediate region (between the two placental sectors). Each section was squeezed with a potato ricer to extract the juice, which was frozen until analysis. For analysis, the juice was diluted 10-fold with distilled water, and then centrifuged at 3000 × g for 10 min before being filtered through a 0.45 μm filter. Twenty microliters of the filtrate were then injected into a high-performance liquid chromatograph (HPLC) (LC-10ADvp; Shimadzu, Japan) fitted with a refractive index detector (RID-10A; Shimadzu Inc.) and equipped with a Shim-pack analytical column (SCR-101C; Shimadzu) at 80°C. Sucrose, glucose, and fructose standards were injected at 20 g·L⁻¹ into the HPLC before injection of the filtrates. Since sweetness is a subjective sensory phenomenon and varies with sugar concentrations, relative sweetness is evaluated using the scale described by Elmstrom and Davis (1981), with sucrose assigned a value of 100, fructose 140–175 and glucose 60–75.

Cell size measurement

Three disks, each about 10 mm thick, were excised from the maximum transverse diameter toward the calyx end of each of three fruits from each treatment (Fig. 1). Using a sharp table knife, sections approximately 10 mm × 10 mm × 10 mm were taken from the central, intermediate, and outer regions of the disk. Each section was fixed, dehydrated, embedded in paraffin, and cut into 15 μm sections. The sections were stained with hematoxylin and eosin solutions and observed under a microscope. The images were photographed and then digitized using a scanner (EPSON GT9300-UF). Maximum cell diameter and cell area of all cells in a

**Fig. 1.** Regions sampled for analysis of sugar content and cell size.
2 mm² section of non-vascular tissue were measured using the NIH Image program, as described previously (Fukuoka et al., 2007).

Results

The daily maximum air temperatures around fruits were 25–35°C in the control, 5–17°C higher in PE, while 2–5°C lower in IRB and BNM (Fig. 2A). The fruit surface temperature difference between the treatments was evident on a clear day (Fig. 2B). In PE, maximum fruit temperature reached about 50°C, and was approximately 7°C higher than the control. The temperatures in IRB and BNM were 5 and 15°C lower than the control, respectively.

Mean fruit weights in PE were about 9 and 11 kg at 30 and 48 DAA, respectively, and were approximately 20–30% greater than those of control fruit of comparable age (Fig. 3). Conversely, plants in IRB and/or BNM produced fruits of lower mean weights than control fruits.

A significant increase in the number of large cells, especially in the intermediate and outer regions of fruit, was observed in PE as compared with the control, but film-covering with IRB and/or BNM brought an increasing number of small cells (Fig. 4). Mean cell lengths in the intermediate and outer regions of fruit in PE were 610 and 660 μm at 40 DAA, respectively, and were approximately 30% longer than those of control fruit. On the other hand, the lengths of IRB and BNM fruits ranged from 400 to 450 μm, and were approximately 7–20% shorter than those of control fruit. Changes in cell area among the experimental plots were similar to cell length.

Sucrose contents of fruit in PE and control did not differ significantly; less glucose and fructose were detected in PE fruit than in control fruit of comparable age (Fig. 6). Fructose and sucrose contents tended to be higher in IRB fruit than in control fruit, especially at 48 DAA, except for fructose content in the outer portion of fruit, although no statistical difference was observed in glucose contents between these treatments. Here, it should be noted that BNM covering had less effect on sugar accumulation than IRB, regardless of the lowest temperature condition among the treatments.

The texture and sweetness of the edible flesh largely determine watermelon quality. Since sweetness is a subjective sensory phenomenon and varies with sugar concentrations, we evaluate relative sweetness. The results revealed that relative sweetness at 48 DAA was highest in IRB fruit in all regions of the fruit, but the values were 10–35% lower in PE than in control fruit (Fig. 7).

Discussion

The developmental processes of fruits in higher plants can be divided into three distinct phases (Gillaspy et al., 1993; Higashi et al., 1999). The earliest phase involves the development of the ovary. In the second phase, fruit growth is due primarily to cell division, whereas in the third phase, growth occurs mainly by cell enlargement. Fruit growth then stops and maturation begin and so the
Fig. 4. Differences of cell shape and size of fruit in the control (A, E, I), polyethylene film (B, F, J), infrared-ray-blocking film (C, G, K) and black nylon mesh (D, H, L). Upper row, central region; Middle row, intermediate region; Lower row, outer region. Fruits were collected at 40 DAA.

Fig. 5. Effect of different tunnel film materials on cell length and cell area of the fruit at 40 DAA. A, control; B, polyethylene film; C, infrared-ray-blocking film; D, black nylon mesh. Vertical bars indicate SE (n = 3).

Fig. 6. Effect of different tunnel film materials on sugar contents during maturation. ●, control; ○, polyethylene film; □, infrared-ray-blocking film; △, black nylon mesh. Vertical bars indicate SE (n = 3).
final fruit size is usually determined by the end of the third developmental phase. In our study, treatments were given during the latter half of the fruit developmental period and this stage corresponded to the duration from the middle of the third phase to maturation in watermelon (Elmstrom and Davis, 1981; Kano, 1991).

Our results revealed that cell enlargements in the intermediate and outer regions of fruit were accelerated by PE film-covering during the latter half of the fruit developmental period. Conversely, these cell enlargements were restricted by IRB film-covering. Cultivated melon fruit at higher temperature (Kano and Fukuoka, 2006; Suzuki et al., 1993) and heat treatment of melon fruit itself (Kano, 2006) increase the number of larger cells and produce larger fruit. Masuda (1970) reported that cells grew larger in melons ‘Earl’s Favorite’ with anthesis on 6 August than in fruit with anthesis on 20 June, and grew larger when night temperatures rose from 10 to 30°C. These results suggest that heat treatment of watermelon fruit during the latter half of the fruit developmental period accelerates cell enlargement in the intermediate and outer regions of fruit, resulting in rapid fruit growth in this period.

In Satsuma mandarin oranges, total soluble solid and sugar contents in juice are higher in fruits developed under 23°C than those under 30°C during the latter half of the fruit developmental period (Utsunomiya et al., 1982). Kobayashi et al. (1967), in an experiment in which ‘Delaware’ grapes were grown under various temperatures ranging from 15 to 30°C during the latter half of the fruit developmental period, found that the soluble solids content decreased in fruits developed under high temperatures. Kano (2004) conducted an experiment in which watermelon fruits were covered with different film materials during the latter half of the fruit developmental period, and found that sugar content was highest in the shade, but lowest under PE film-covering. In our study, higher sugar content could be produced by covering with IRB film instead of PE and the relative sweetness in all regions of fruit was higher in the former than the latter. In Japanese persimmon, the contents of soluble solids and sugars increased in fruits developed under low temperatures (Chujo et al., 1972); therefore, it seems that sugar accumulation during the latter half of the fruit developmental period largely depends on fruit temperatures in watermelon, and that high sugar content can be produced by lowering fruit temperature with IRB film.

Most fruits can be classified as utilization sinks during fruit development because of their high metabolic activity; they then become storage sinks during the maturation period (Gillaspy et al., 1993; Sonnewald and Willmitzer, 1992). The mean cell volume of strawberry fruit increases slowly during active cell division, but rises rapidly and linearly for 10 days after cell division is halted (Guiwen and Breen, 1992). Sucrose content in strawberry fruit increases from 25 to 35 DAA (Ofosu-Anim and Yamaki, 1994). In watermelon, fruit size increased rapidly for 20 DAA and then slowed down (Kano, 1991), and sucrose was not detected before 20 DAA, after which sucrose was accumulated proceed (Elmstrom and Davis, 1981; Porter et al., 1940). These results suggest that the relationship between cell enlargement and sugar accumulation in fruit is a mirror image of each other.

Translocated sugars in fruit are partly accumulated as soluble sugars and the rest is used for structural materials, such as the cell wall and for respiration (Kawabata et al., 2002). Whiting (1970) reported that sucrose was the carbohydrate source for the construction of the cell structure and for energy provision in fruit. Furthermore, Meyer and Anderson (1952) reported that glucose and fructose, the components of sucrose, were the most common substrates for respiration and the rise of temperature resulted in an increase in the respiration rate. In our study, as shown above, fruit growth was accelerated by fruit heating during the latter half of the fruit developmental period. Hence, we consider that the
observed decreases in sugar content in PE fruit largely depend on high sugar consumption, owing to both increased respiration in fruit and the rapid incorporation of sugars into the cell structure caused by vigorous fruit growth under high temperature. In IRB fruit, sugar consumption remained low due to the reduction of respiration and lower incorporation of sugars into the cell structure by hindering fruit growth, so that sugar levels remain high. In experiments in cucumber (Kawabata and Sakiyama, 1998) and tomato (Kawabata et al., 2002), fruit growth depression by mechanical restriction promoted sugar accumulation.

Here, the significant fact to be noted was that covering with BNM film had less effect on sugar accumulation than IRB, regardless of the lowest temperature condition among the experimental treatments. BNM used in this experiment reduced light intensity by about 60% and covered the leaves around fruit. Oe et al. (1994) pointed out that Brix percentage in watermelon fruit fell after defoliation treatment around fruit. Although the amount of photosynthetic products was not measured in our study, shading leaves around fruit might reduce these elements. We consider that decreased sugar accumulation in BNM is mainly due to a lower amount of photosynthetic products in leaves around fruit. Eventually, the sugar content of IRB fruit become greater than that of BNM fruit.

**Literature Cited**


