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Development of a New Melon Cultivar 'Ibaraking' with High Fruit Growth Ability under Low Temperature Conditions, High Total Soluble Solid Content, and Resistance to Fusarium Wilt

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Abstract

'Ibaraking' is a new F_1 melon (*Cucumis melo* L.) cultivar with green-fleshed fruit, high fruit growth ability under low-temperature conditions, high total soluble solid (TSS) content, and a long shelf life. It was developed from a cross between 2 parental lines P2 and P32 at the Plant Biotechnology Institute, Ibaraki Agricultural Center, Ibaraki, Japan. The parental line, P2, was fixed by self-pollination of a line selected from somaclonal variants of a cultivar 'Andes,' while another line, P32, was fixed by self-pollination of a cultivar 'Earl's Seine Natsu 2.' The fruit characteristics of 'Ibaraking' were compared with those of the common melon semi-forcing culture cultivars 'Andes-5' and 'Otome,' from 2005 to 2007, in semi-forcing culture by creeping cultivation. In 'Ibaraking,' low-temperature fruit growth ability and external appearance were equal to those of 'Otome' and exceeded those of 'Andes-5,' while the TSS content was similar to that of 'Andes-5' and exceeded that of 'Otome.' With regard to shelf life, 'Ibaraking' was superior to 'Otome' and equal to 'Andes-5.' In response to Fusarium wilt of melon, the most severe melon disease, 'Ibaraking' demonstrated resistance to races 0 and 2. These responses were identical to those of 'Andes-5' and 'Otome.' Therefore, we conclude that 'Ibaraking' appears a more suitable cultivar in semi-forcing culture by creeping cultivation in Ibaraki prefecture, Japan.

Discipline: Horticulture
Additional key words: *Cucumis melo*, Fruit firmness, Shelf life

Introduction

Melon (*Cucumis melo* L.) is one of the most important dessert cucurbits worldwide. In Japan, Ibaraki prefecture is the highest yielding production area, where 'Andes' (Sakata seed Corp., Japan) and 'Otome' (Takii seed Co. Ltd., Japan) cultivars are commonly grown. These cultivars have green-fleshed fruit and are cultivated in semi-forcing culture by creeping cultivation. However, they also often demonstrate inferior fruit growth or total soluble solid (TSS) content under low-temperature conditions in semi-forcing culture (Kaneko et al. 2005). Therefore, the popularity of these cultivars with farmers and consumers has declined, and development of a replacement is urgently required to maintain melon production in the area. In particular, fruit growth ability under low-temperature conditions is an important characteristic in the melon semi-forcing cultivar. In a previous report, low-temperature germinability was reported as an indicator for selecting melon lines with fruit growth ability under low-temperature conditions, while lines with high fruit growth ability were developed from somaclonal variations (Ezura et al. 2005).

Fusarium wilt of melon caused by *Fusarium oxysporum* f. sp. *melonis* is considered the most severe melon disease. Once the disease colonizes a field, the pathogen survives in the soil because crop residues and roots of most crops, grown in rotation with melons, increase these persistent pathogenic populations. *Fusarium* strains are divided into 4 races (0, 1, 2, and 1,2) based on their pathogenicity on a set of differential genotypes. Races 0 and 2 were generally observed in Japan, while resistant cultivars are effective for controlling such soilborne diseases in melons (Matsumoto et al. 2011, Namiki et al. 1998).

We developed a parental line from somaclonal variations in low-temperature germinability and Fusarium wilt

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resistance. The newly developed melon cultivar, 'Ibaraking', has high fruit growth ability under low-temperature conditions, high-quality green-fleshed fruit, including high TSS content, and resistance to the Fusarium wilt of melon races 0 and 2. Here we describe the breeding process and main fruit characteristics of the new melon cultivar 'Ibaraking.'

Materials and Methods

1. Parental line and breeding process

The parental line, P2, was fixed by self-pollination of a line selected from somaclonal variants of a cultivar 'Andes' with low-temperature germinability as an indicator of fruit growth ability under low-temperature conditions, according to the method of Ezura et al. (2005). It has high fruit growth ability under low-temperature conditions and resistance to Fusarium wilt races 0 and 2. Another parental line, P32, was fixed by self-pollination of a cultivar 'Earl's Seine Natsu 2' (Yae Nogei Co. Ltd., Japan) and is particularly palatable. These parental lines were used for the cross in 2003.

The F_1 cross combination was evaluated and selected in the experimental field and at farm sites from 2004 to 2007. Furthermore, 2 common semi-forcing cultivars in Ibaraki prefecture, 'Andes-5' and 'Otome,' were used for contrast with the F_1 cross combination.

2. Evaluation of resistance to Fusarium wilt

The response of 'Ibaraking' to Fusarium wilt races was compared with those of 'Andes-5' and 'Otome' and the parental lines P2 and P32. For the inoculation test, the fungal strains JCM 9889; Mel 02221; JCM 9288; and Fom 142-S1, which are classified as races 0; 1; 2; and 1,2, respectively (Matsumoto et al. 2011, Namiki et al. 1998, 2000), were used. Inoculation was performed using a root dip method (Matsumoto et al. 2011). Each fungal strain was cultured in 100 mL of potato dextrose broth (PDB) in 300-mL flasks on a rotary shaker (ca. 120 rpm) for 1 week at 25°C, whereupon the culture was passed through a 2-ply gauze. The spore concentration, determined using a hemocytometer, was adjusted to the appropriate density (10^7 spores/mL) by dilution with sterile distilled water. For artificial inoculation, seeds of the tested plants were sown into garden soil in plastic trays and grown in a growth chamber at 26-30°C and seedlings with a fully expanded first true leaf were removed from the soil. Their roots were washed using tap water and dipped in the conidial suspension for 15 s. Inoculated seedlings were then transplanted to fresh garden soil in new plastic pots and cultivated in a growth chamber at 23°C (16 h photoperiod). The disease severity was evaluated 21 days after inoculation according to no symptoms or plant death. A total of 10 plants were evaluated for each cultivar.

3. Evaluation of fruit characteristics

The fruit characteristics of 'Ibaraking' were compared with those of 'Andes-5' and 'Otome' in semi-forcing culture by creeping cultivation in 2005-2007. Seedlings were planted in the greenhouse at 55-cm intervals on February 7 and 10 and on January 18 in 2005, 2006, and 2007, respectively. Plants were topped at the 3^{rd} to 5^{th} node, 2 lateral shoots trained to creep were grown as the main stems, and 1 to 2 fruit per stem were set at the 11^{th}-15^{th} nodes. In total, 3 to 4 fruit were set per plant and mature fruit were harvested approximately 60 days after pollination. In all, 18-40 fruit per cultivar were evaluated each year. The means of weighing the fruit, TSS content, netting density, and netting thickness were determined using ripened melons, while TSS content was determined from the fruit juice extracted from sarcocarp of the equatorial region using a hand refractometer (ATC-1E, Atago Co. Ltd., Japan) and expressed as Brix. The netting density and netting thickness were scored on a 5-point scale and the results were as follows: density; 0 = no netting, 3 = typical 'Andes' type (some of the epidermis was covered with netting), 4 = most of the epidermis was covered with netting, and thickness; 0 = no netting, 3 = typical 'Andes' type (thick netting), 4 = typical Earl's type (very thick netting). Fruit firmness was measured to evaluate the shelf life of the melon according to the method of Johnstone et al. (2008). The harvested fruit were stored at 25°C for 1 and 7 days to allow the fruit to ripen. After storage, the fruit firmness was measured at 3 paired points along the equatorial region of the same fruit using a fruit hardness tester (KM-1, Fujiwara Factory, Japan) fitted with a 12-mm plunger and expressed as kilograms. In each cultivar, 8-20 fruit were evaluated for each period of stored days in 2007 and 2008. Analysis of variance (ANOVA) and tests of significance for all traits were performed, and a Tukey's honestly significant difference (HSD) post-hoc test was used for comparison. Analysis was performed using JMP statistical software (ver. 9.0.0; SAS Institute Inc., USA).

Results and Discussion

1. Breeding and selection of 'Ibaraking'

The parental lines P2 and P32 were fixed in 1999 and 2002 by self-pollination respectively and used for crossing in 2003 (Fig. 1). In 2004, 30 F_1 hybrids from crosses between several parental lines, including P2 and P32, were grown in a greenhouse in semi-forcing culture, and line H421 was selected based on field performance and renamed Seiken-Ko-14. In 2005, 2006, and 2007, the line was evaluated in semi-forcing culture by creeping cultivation for agricultural characteristics, renamed Hitachi-Ko-3, and its local adaptability was evaluated at farm sites of Hokota city, Ibaraki prefecture, in semi-forcing culture by creeping cultivation in 2006 and 2007. Based on these data, we selected

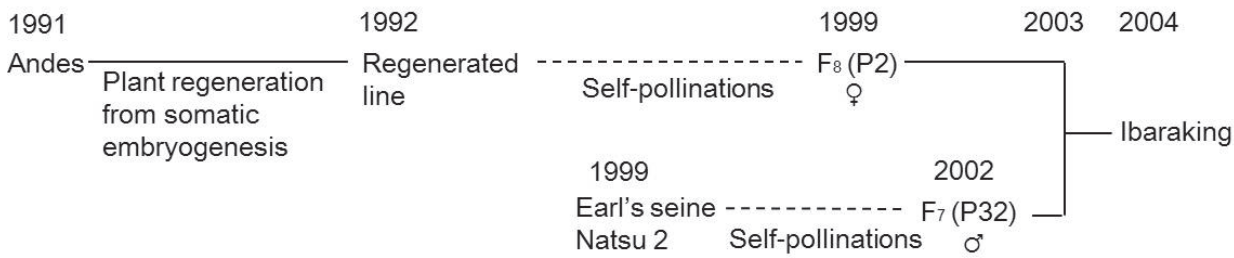

Fig. 1. The pedigree of 'Ibaraking' and parental lines

Fig. 2. Mature fruit of 'Ibaraking' (Bar = 2cm)

the line and subsequently officially registered 'Ibaraking' with the Ministry of Agriculture, Forestry and Fisheries of Japan. In 2010, 'Ibaraking' was assigned the cultivar registration number 19804 (Fig. 2).

2. Resistance to Fusarium wilt

For races 0 and 2, none of the seedlings of 3 cultivars and parental line P2 presented any symptoms. In contrast, for races 1 and 1,2; all seedlings of the 3 cultivars and both parental lines died (Table 1). Thus, 'Ibaraking' was considered resistant to race 0 and 2, and the resistance was provided by the parental line P2. The resistant reaction of 'Ibaraking' resembled that of 'Otome' and 'Andes-5.'

Table 1. Pathogenicity of *Fusarium oxysporum* f. sp. *melonis* races to three melon cultivars and parental lines

Cultivar or line name	Mean disease symptoms[a]			
	Race			
	0	1	2	1,2
Ibaraking	NS	PD	NS	PD
Otome	NS	PD	NS	PD
Andes-5	NS	PD	NS	PD
P2	NS	PD	NS	PD
P32	PD	PD	PD	PD

[a] NS = no symptoms and PD = plant death. n= 10.

3. Fruit characteristics

Significant differences among the cultivars were also observed in fruit weight, TSS content, and netting density but not netting thickness. In addition to significant differences among cultivars and years, there were significant year by cultivar interactions for fruit weight, TSS content, and netting density (Table 2). In 'Ibaraking,' the fruit weight resembled that of 'Otome' and exceeded that of 'Andes-5.' TSS content resembled that of 'Andes-5' and exceeded that of 'Otome,', while the netting density was similar to that of 'Otome' and exceeded that of 'Andes-5' (Figs. 3, 4). From the fruit weight and netting density results, the fruit growth ability under low-temperature conditions and the external appearance of 'Ibaraking' appeared similar to that of 'Otome' and superior than that of 'Andes-5.'

For fruit firmness, although no significant differences among cultivars were observed 1 day after harvest (DAH), significant differences were observed at 7 DAH (Table 3). The fruit firmness of 'Ibaraking' exceeded that of 'Otome' at 7 DAH. The firmness was 1.19, 1.15, and 1.12 kg at 1 DAH, and 0.67, 0.89, and 0.83 kg at 7 DAH in 'Otome,' 'Andes-5,' and 'Ibaraking,' respectively (Fig. 5). The shelf life of 'Ibaraking' was accordingly concluded to be equivalent to that of 'Andes-5' and superior to that of 'Otome.' For fruit distribution, the shelf life of the ripened fruit is

Table 2. Result of two-factor analysis of variance (ANOVA) for several parameters among the three melon cultivars from 2005 to 2007

Parameter		d.f.	Mean square	*F*	*P*
Fruit weight (g)	Cultivar	2	1835266	34.14	<0.001
	Year	2	6350689	118.13	<0.001
	Year × cultivar	4	838869	7.80	<0.001
	Error	311	26881		
Total soluble solids content (°Brix)	Cultivar	2	70.33	15.44	<0.001
	Year	2	439.45	96.45	<0.001
	Year × cultivar	4	68.04	7.47	<0.001
	Error	309	2.28		
Netting density scale	Cultivar	2	13.34	72.21	<0.001
	Year	2	18.46	99.88	<0.001
	Year × cultivar	4	2.54	6.88	<0.001
	Error	302	0.09		
Netting thickness scale	Cultivar	2	11.60	1.96	0.14
	Year	2	14.79	2.49	0.08
	Year × cultivar	4	10.47	0.88	0.47
	Error	302	2.96		

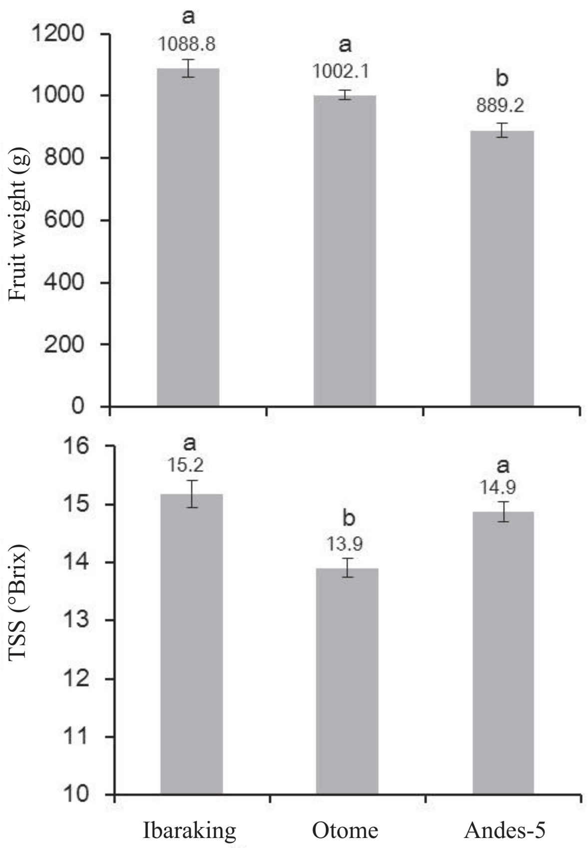

Fig. 3. Fruit weight and total soluble solid content of the 3 melon cultivars

The data shown are the means for 3 years (2005-2007) (n = 3). Identical superscript letters denote no significant differences between cultivars after Tukey's honestly significant difference (HSD) post-hoc paired comparisons for pooled data for each cultivar. Error bar = S.E.

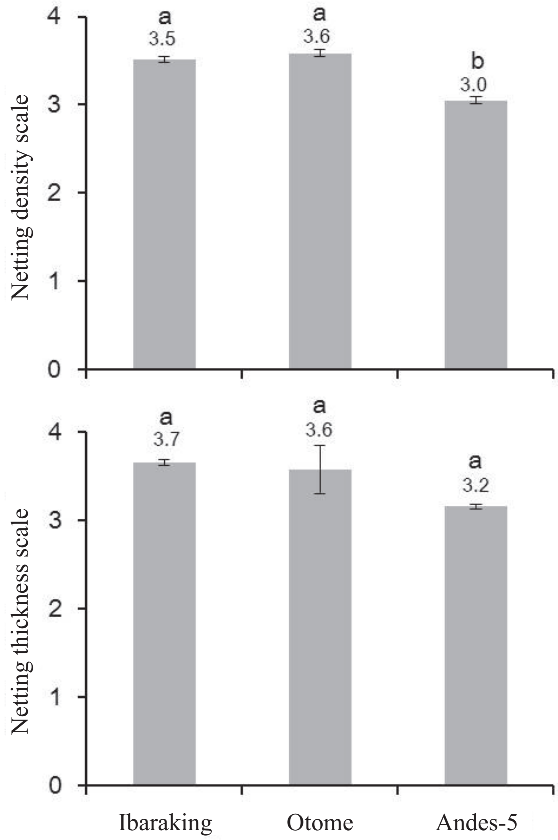

Fig. 4. Netting density and thickness scale of 3 melon cultivars

The data are the means for 3 years (2005-2007) (n = 3). Identical superscript letters denote no significant differences between cultivars after Tukey's HSD post-hoc paired comparisons for pooled data for each cultivar. Error bar = S.E.

Table 3. Result of two-factor ANOVA for fruit firmness several days after harvest (DAH) in three melon cultivars from 2005 to 2007

DAH		d.f.	Mean square	F	P
1	Cultivar	2	0.18	1.72	0.18
	Year	1	22.56	42.87	<0.001
	Year × cultivar	2	0.10	0.97	0.38
	Error	76	0.05		
7	Cultivar	2	0.63	9.87	<0.001
	Year	1	2.65	82.12	<0.001
	Year × cultivar	2	0.24	3.82	0.02
	Error	81	0.03		

important and that of 'Andes-5' was relatively long for Japanese melons. We propose accordingly that it is as suitable for Japanese distribution as 'Andes-5.'

In the previous study, the adaptation of 4 melon cultivars in semi-forcing culture by creeping cultivation in Ibaraki prefecture was evaluated, and 2 cultivars, 'Otome' and 'Andes-5,' were suggested as suitable for semi-forcing culture (Kaneko et al. 2005). In this study, most fruit characteristics of 'Ibaraking' such as fruit growth ability under low-temperature conditions, TSS content, external appearance, and shelf life were similar to or superior to that of the other 2 cultivars. Therefore, 'Ibaraking' appeared a more suitable cultivar in semi-forcing culture by creeping cultivation in Ibaraki prefecture and would contribute to melon production.

4. Seed availability

Seeds of 'Ibaraking' are multiplying every year and available from the Plant Biotechnology Institute, Ibaraki Agricultural Center for scientists, and Horticultural Ibaraki Promotion Association (Mito, Ibaraki, Japan) for Ibaraki prefectural farmers.

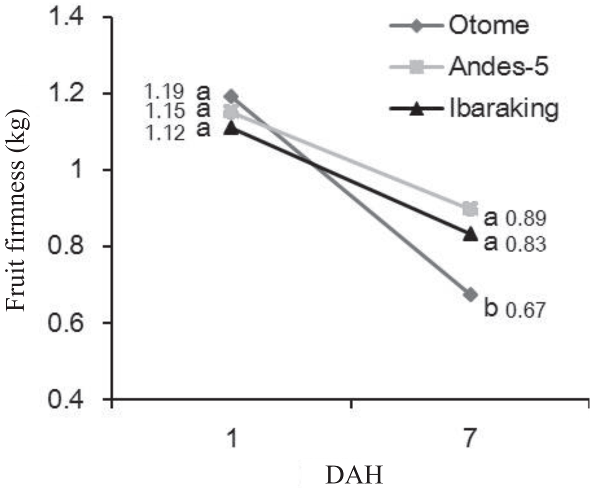

Fig. 5. Fruit firmness of the 3 melon cultivars at 1 and 7 days after harvest respectively

The data are the means for 3 years (2005-2007) (n = 3). Identical superscript letters denote no significant differences between cultivars after Tukey's HSD post-hoc paired comparisons for pooled data for each cultivar.

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