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Evaluation of the juice brix of wild sugarcane (*Saccharum spontaneum*) indigenous to Japan

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ABSTRACT

Modern sugarcane cultivars are derived from the interspecific crossing between *Saccharum officinarum* and wild sugarcane, *Saccharum spontaneum*. The introgression of valuable characteristics from wild sugarcane is recognized as extremely important, but this process typically requires long-term effort over multiple generations of backcrosses owing to the low sugar content of the initial interspecific hybrids. In this study, we aimed to identify Japanese wild sugarcane with high juice brix in order to promote effective interspecific crossing of sugarcane. Sixty-four accessions from the Nansei Islands and 70 accessions from the Honshu were evaluated for juice brix. Wild sugarcane with high juice brix were demonstrated to exist among wild sugarcane indigenous to the Honshu. A significant difference was observed between the median juice brix values of wild sugarcane of the Nansei Islands and those of the Honshu. The relationship between juice brix and stem traits was then examined in 20 wild sugarcane, 10 each from the Nansei Islands and the Honshu. The reproducibility of juice brix value in both experiments was confirmed. In contrast to juice brix, stem traits, such as length, diameter, and volume, were typically smaller in wild sugarcane from the Honshu. Moreover, a negative correlation was observed between the index of stem volume and juice brix. In this study, we identified outstanding wild sugarcane with high juice brix. Using germplasms from the identified wild sugarcane in interspecific crossing could contribute to the increases in both yield and sugar content.

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Sugarcane is cultivated widely in the tropical, subtropical, and temperate zones for use in sugar production. Modern sugarcane cultivars (*Saccharum* spp. hybrid) are derived from interspecific crossing between *Saccharum officinarum* and its relatives (Daniels & Roach, 1987). In particular, the wild sugarcane, *Saccharum spontaneum* is known to be a key antecedent to modern sugarcane cultivars, with outstanding characteristics such as tolerance to environmental stresses, resistance to diseases, and ratooning ability.

As shown in Rao et al. (1973), it is generally recognized that the sugar content of wild sugarcane is lower than that of *S. officinarum* and its hybrid clones. Initial interspecific hybrids with wild sugarcane therefore must be crossed back to *S. officinarum* or previously backcrossed clones to achieve sufficiently high sugar content. This process is historically referred to as 'nobilization' (Bremer, 1961). Though the introgression from wild sugarcane is recognized as extremely important, accomplishing this requires

a long-term effort. Therefore, only a small number of wild sugarcane clones already shown to be successful are utilized in current breeding programs, and the researches to broaden the genetic base have been conducted (Brown et al., 1969; Nagatomi, 1985; Roach, 1977; Wang et al., 2008).

Wild sugarcane grow naturally from Asia to parts of Oceania and Africa (Panje & Babu, 1960). There are native populations of wild sugarcane in Japan along the coastline of the Pacific Ocean (Nagatomi et al., 1984, 1985; Sakaigaichi et al., 2007; Shimoda et al., 2000), and wild sugarcane clones collected from these populations have been conserved as a genetic resource. Nagatomi and Degi (2007) report that wild sugarcane indigenous to the Honshu have high juice brix. These wild sugarcane with high sugar content could contribute to breakthroughs in interspecific crossing by increasing the efficiency of nobilization. However, scientific information on this topic is limited because few wild sugarcane with high sucrose content have been identified.

We thus sought to identify high-brix Japanese wild sugarcanes with potential to improve the yield and sugar content of interspecific sugarcane hybrids. To this end, we first identified wild sugarcanes with high brix from Japanese native plants. After screening the germplasm of over 130 wild sugarcanes, we selected a subset in which we investigated the relationship between juice brix and the visible traits of the stem wherein sucrose accumulates.

2. Materials and methods

2.1. Experiment 1: evaluation of the juice brix of Japanese wild sugarcanes

This study was conducted at the genebank field of the NARO Kyushu Okinawa Agricultural Research Center in Nishinoomote, Kagoshima prefecture, Japan (30°44' N, 131°04' E) in 2007 and 2008. The soil of the field is Andosol. A total of 134 Japanese wild sugarcanes conserved as a sub-bank of the National Institute of Agrobiological Sciences (NIAS) were tested in Exp. 1. These wild sugarcanes, their accession numbers, and their collection sites are described in accordance with the NIAS Genebank database (Figure 1 and Table 1). A geo-biological demarcation exists between the Nansei Islands and the Japan mainland, caused by the border between the subtropical and temperate zones. We therefore chose 64 accession numbers of wild sugarcane from the Nansei Islands, where most sugarcanes grow, and 70 others from the Honshu, where very little sugarcane grows.

Wild sugarcanes were conserved as ratoon crops by cutting shoots every April. The space between hills was about 110 cm. No fertilizer was applied so as to preclude vigorous

growth. The investigations of juice brix were conducted on 17 December 2007, and 28 November 2008. Five healthy stems from each accession were selected to measure the juice brix. Cane juice was squeezed from the middle of the stem length, and juice brix was investigated using a hand-held refractometer (ATAGO, MASTER-PA). Stem length was defined as follows: in flowering cane, it was measured from the base to the node of the 9th expanded leaf; in non-flowering cane, it was measured from the base to the node of the 5th expanded leaf.

2.2. Experiment 2: relationship between juice brix and stem traits

In Exp. 1, our investigation focused on wild sugarcanes conserved in the genebank field. In Exp. 2, we planted wild sugarcanes in the experimental field of the station, and examined juice brix and stem traits.

Ten wild sugarcane accessions from the Nansei Islands (JW70, JW90, JW223, JW259, JW294, Iriomoe34, 99JW-10, 99JW-15, 99JW-18, and 99JW-23), and ten accessions from the Honshu (JW529, JW545, JW559, JW577, JW614, JW622, JW634, JW640, JW663, and 06JW-3), were chosen as a subset for Exp. 2. Wild sugarcanes tested in Exp. 2 were selected to cover the variation in juice brix observed in Exp. 1. NiF8, a leading cultivar for sugar mill use, and KRfO93-1, a cultivar for forage use, were also studied for comparison.

Five suckers separated from the stubble of each wild sugarcane accession were planted in Jiffy pots on 2 April 2012. Five buds each cut from healthy stems of NiF8 and KRfO93-1 were also planted in Jiffy pots. The pots were transplanted into the field on 8 April, with a row space of

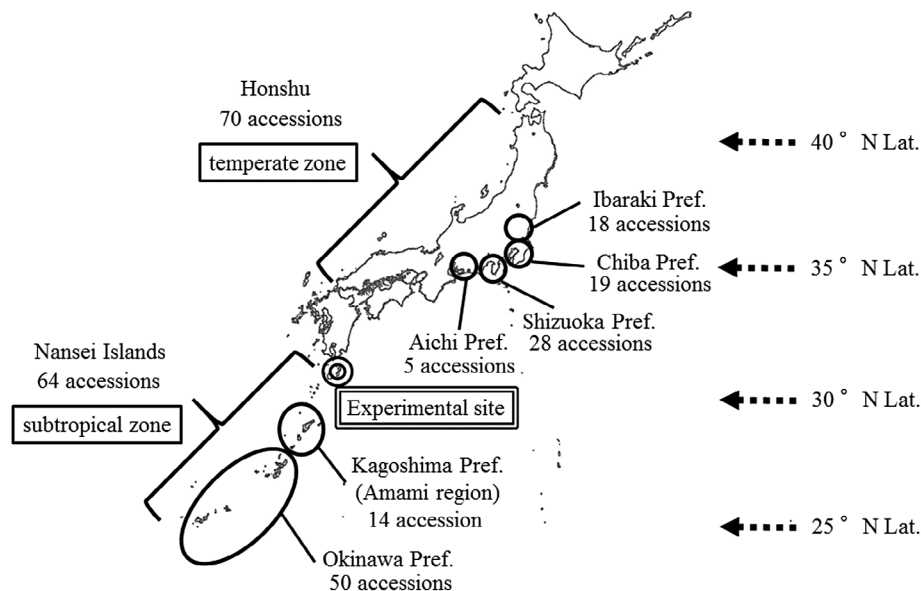


Figure 1. Collection sites of Japanese wild sugarcanes tested in Exp. 1.

Table 1. Juice brix of Japanese wild sugarcanes in Exp. 1

Collection site		Accession name	JP number	Juice brix (%)			Collection site		Accession name	JP number	Juice brix (%)			
				2007	2008	Average					2007	2008	Average	
Nansei Islands	Okinawa Pref.	JW58	174,472	10.5	8.8	9.6	Honshu	Aichi Pref.	06JW-1	232,241	19.4	14.2	16.8	
		JW69	174,473	11.6	6.6	9.1			06JW-3	232,243	18.0	17.5	17.7	
		JW70	152,907	10.5	7.4	9.0			06JW-4	232,244	18.9	11.9	15.4	
		JW75	174,474	10.4	9.4	9.9			06JW-5	232,245	17.1	15.2	16.2	
		JW79	174,475	10.6	7.6	9.1			06JW-6	232,246	15.2	12.7	13.9	
		JW88	174,476	8.9	8.2	8.6			Shizuoka Pref.	JW609	173,953	15.8	13.6	14.7
		JW90	174,477	8.8	6.6	7.7				JW613	174,513	15.0	15.4	15.2
		JW111	168,290	7.5	7.3	7.4				JW614	174,514	18.1	15.1	16.6
		JW131	174,480	6.6	6.6	6.6				JW617	173,954	15.6	14.2	14.9
		JW195	174,481	6.0	7.1	6.5				JW622	174,516	15.2	9.7	12.4
		JW204	174,482	6.9	7.0	6.9				JW625	173,955	8.5	8.7	8.6
		JW223	174,483	15.4	14.4	14.9				JW632	174,517	15.2	16.0	15.6
		JW233	168,185	6.8	6.7	6.8				JW634	173,958	18.3	16.3	17.3
		JW258	168,203	9.2	9.8	9.5				JW636	174,518	16.4	16.8	16.6
		JW259	174,484	9.9	13.0	11.5				JW637	174,519	18.5	15.8	17.1
		JW286	168,220	9.3	11.0	10.2				JW639	173,960	16.3	16.0	16.2
		JW289	174,487	8.1	11.1	9.6			JW640	174,520	19.6	16.6	18.1	
		JW293	168,223	6.2	4.8	5.5			JW641	173,961	19.4	15.0	17.2	
		JW294	168,224	5.8	5.7	5.8			JW642	174,521	17.1	15.2	16.1	
		Iriomote1	175,501	5.8	6.0	5.9			JW646	173,962	11.9	18.0	14.9	
		Iriomote2	175,502	6.6	6.4	6.5			JW649	173,963	14.5	16.4	15.5	
		Iriomote3	175,503	6.0	11.0	8.5			JW650	174,525	17.1	18.1	17.6	
		Iriomote4	175,504	6.3	6.3	6.3			JW651	174,526	13.3	16.8	15.0	
		Iriomote5	175,505	10.0	10.4	10.2			JW652	174,527	19.4	17.8	18.6	
		Iriomote6	175,506	9.2	11.5	10.4			JW659	174,529	15.9	10.6	13.2	
		Iriomote7	175,507	10.2	10.3	10.2			JW660	173,965	14.0	11.2	12.6	
		Iriomote9	175,509	6.4	4.6	5.5			JW661	173,966	13.0	13.2	13.1	
		Iriomote10	175,510	10.0	7.5	8.8			JW662	174,530	9.0	14.8	11.9	
		Iriomote11	175,511	10.7	7.3	9.0			JW663	173,967	9.5	11.5	10.5	
		Iriomote12	175,512	10.8	6.8	8.8			JW666	174,532	15.9	16.1	16.0	
		Iriomote13	175,513	11.8	8.0	9.9			JW667	173,969	16.2	14.1	15.2	
		Iriomote14	175,514	8.6	7.5	8.1			JW669	174,533	9.4	15.5	12.4	
		Iriomote15	175,515	6.4	7.0	6.7			JW674	173,971	18.0	15.0	16.5	
		Iriomote16	175,516	10.8	5.9	8.3			Chiba Pref.	JW544	173,920	15.4	13.7	14.5
		Iriomote17	175,517	10.6	10.8	10.7				JW545	173,921	14.0	15.8	14.9
		Iriomote18	175,518	4.0	10.6	7.3				JW547	173,922	12.9	15.8	14.3
		Iriomote19	175,519	8.6	9.9	9.3				JW548	174,497	5.8	7.4	6.6
Iriomote21	175,521	7.9	8.1	8.0	JW552	173,925	17.9	12.9		15.4				
Iriomote22	175,522	9.6	5.8	7.7	JW553	174,499	15.6	9.8		12.7				
Iriomote23	175,523	5.7	6.6	6.1	JW554	173,926	16.0	12.6		14.3				
Iriomote24	175,524	5.4	9.3	7.3	JW555	173,927	12.6	14.0		13.3				
Iriomote25	175,525	7.6	9.6	8.6	JW556	174,500	14.9	13.0		13.9				
Iriomote26	175,526	8.2	7.5	7.9	JW557	174,501	18.6	11.9		15.2				
Iriomote27	175,527	7.2	8.1	7.6	JW558	173,928	18.2	16.1		17.2				
Iriomote28	175,528	6.1	7.8	7.0	JW559	174,502	8.1	7.6	7.8					
Iriomote29	175,529	9.8	10.3	10.1	JW570	173,935	17.9	14.6	16.3					
Iriomote30	175,530	8.0	8.6	8.3	JW571	173,936	18.1	14.4	16.2					
Iriomote33	175,533	8.2	8.4	8.3	JW573	173,937	15.5	14.8	15.2					
Iriomote34	175,534	8.4	8.7	8.5	JW574	174,504	17.8	15.2	16.5					
Iriomote37	175,537	8.9	7.7	8.3	JW577	174,505	14.6	14.8	14.7					
Kagoshima Pref. (Amami region)	99JW-9	232,222	11.8	10.8	11.3	JW579	174,506	17.6	14.2	15.9				
	99JW-10	232,223	12.0	8.4	10.2	JW580	173,940	17.6	14.7	16.1				
	99JW-11	232,224	7.4	6.4	6.9	Ibaraki Pref.	JW517	173,905	13.3	13.0	13.2			
	99JW-13	232,226	8.6	7.2	7.9		JW518	173,906	7.3	8.7	8.0			
	99JW-14	232,227	10.1	8.8	9.5		JW519	173,907	9.2	10.3	9.8			
	99JW-15	232,228	5.6	8.0	6.8		JW521	173,909	8.5	8.1	8.3			
	99JW-16	232,229	10.4	9.8	10.1		JW522	173,910	17.0	15.0	16.0			
	99JW-17	232,230	10.3	9.0	9.6		JW523	173,911	8.2	8.3	8.2			
	99JW-18	232,231	7.5	9.6	8.5		JW525	173,912	11.4	14.3	12.9			
	99JW-19	232,232	8.8	9.8	9.3		JW527	173,913	8.6	14.2	11.4			
99JW-20	232,233	11.3	9.6	10.5	JW529		174,491	6.5	8.9	7.7				
99JW-21	232,234	8.9	9.6	9.2	JW530		173,914	9.7	14.6	12.1				
99JW-23	232,236	13.1	11.8	12.4	JW536	174,494	15.3	14.2	14.7					
99JW-26	232,239	13.2	8.3	10.7	JW540	173,918	16.7	14.6	15.6					
Average of Nansei Islands				8.8	8.4	8.6	Average of Honshu				14.5	13.6	14.1	

110 cm and an interhill space of 50 cm. This study was set up in a randomized block design with three replications.

Basal dressing was applied at the rate of 7.2 g N m⁻², 12.0 g P₂O₅ m⁻², and 6.0 g K₂O m⁻² at the time of transplanting. Topdressing was applied at the rate of 9.0 g N m⁻² and 9.0 g K₂O m⁻² on 24 May.

A harvest survey of the crop was conducted on 22 November. Three healthy stems were selected to measure the juice brix and stem traits such as stem length and diameter. Juice brix and stem length were measured as in Exp. 1. Stem diameter was measured as the minor-axis portion of the node in the middle of the stem by digital calipers. Index of stem volume was estimated based upon our measurements of stem length and stem diameter according to the formula 'Index of stem volume = Stem length × (half of Stem diameter)².

2.3. Statistical analyses

Statistical analyses were conducted using the software package (IBM, SPSS ver. 21.0). The median values of juice brix in wild sugarcanes of the Nansei Islands and the Honshu were analyzed by Mann–Whitney U test.

3. Results

3.1. Experiment 1: evaluation of the juice brix of Japanese wild sugarcanes

The average juice brix of Japanese wild sugarcanes in 2007 and 2008 are shown in (Table 1). Among wild sugarcanes of the Nansei Islands, JW293 and Iriomote9 had the lowest juice brix (5.5%), and JW223 had the highest (14.9%). Among wild sugarcanes of the Honshu, JW548 had the lowest juice brix (6.6%), and JW652 had the highest (18.6%).

Box plots of the juice brix in wild sugarcanes of Nansei Islands and the Honshu are shown in Figure 2. The 25th percentile was 7.3% and the 75th percentile was 9.8% for the Nansei Islands. On the other hand, the 25th percentile was 12.5% and the 75th percentile was 16.1% for the Honshu. The median value of the juice brix was also higher in wild sugarcanes from the Honshu (14.9%), than that of the Nansei Islands, (8.5%). The difference between the two median values was significant at 1% level of probability as calculated by Mann–Whitney U test.

3.2. Experiment 2: relationship between juice brix and stem traits

The juice brix measured in Exp. 2 is shown in (Table 2). The juice brix ranged from 7.4% in JW90 to 14.8% in JW223 for the 10 tested wild sugarcanes from the Nansei Islands, and

the average was 9.8%. For the 10 wild sugarcanes from the Honshu, the juice brix ranged from 7.3% in JW559 to 17.3% in 06JW-3 and JW640, and the average was 14.1%. There was a positive correlation between the juice brix measured in Exp. 1 and that in Exp. 2 at 1% level of probability (Figure 3). NiF8, a cultivar for sugar mill use, measured 18.2% and KRf093-1, a cultivar for forage use, measured 15.4%. The

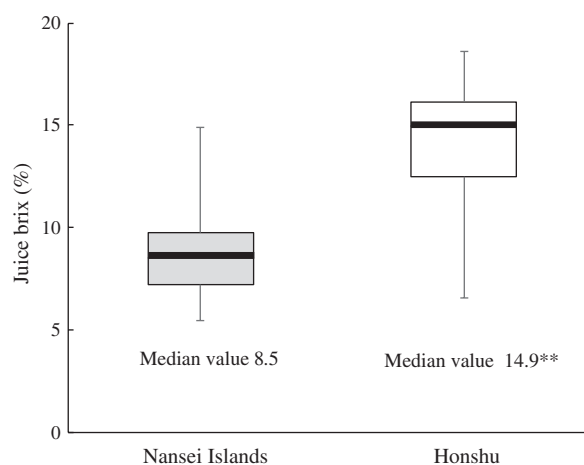


Figure 2. Box plots of the juice brix of Japanese wild sugarcanes in Exp. 1.

The left and right patterns indicate the box plots of wild sugarcanes collected from the Nansei Islands and the Honshu, respectively. The line through the middle of each box indicates the median value. Each box begins at the 25th percentile and ends at the 75th percentile of the data-set. The vertical bars indicate the maximum and minimum values. **indicates a significant difference at 1% level of probability according to the Mann–Whitney U-test.

juice brix of wild sugarcanes such as 06JW-3 and JW640 was comparable to that of NiF8.

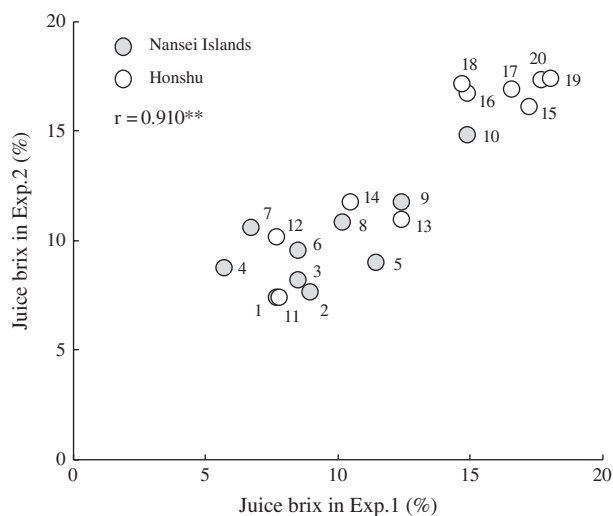
The stem length, stem diameter, and index of stem volume of wild sugarcanes are shown in (Table 2). The stem length ranged from 53 cm in JW223 to 128 cm in Iriomote34 for wild sugarcanes of the Nansei Islands, and the average was 95 cm. The stem length ranged from 53 cm in JW640 to 121 cm in JW622 for wild sugarcanes of the Honshu, and the average was 83 cm. The stem diameter ranged from 0.50 cm for JW223 to 1.00 cm for JW259 in wild sugarcanes of the Nansei Islands, and the average was 0.71 cm. The stem diameter ranged from 0.45 cm in 06JW-3 to 0.73 cm in JW663 in wild sugarcanes of the Honshu, and the average was 0.56 cm.

The index of stem volume ranged from 3.3 in JW223 to 32.1 in JW259 for wild sugarcanes of the Nansei Islands, and the average was 13.1. On the other hand, the index of stem volume ranged from 3.3 in JW545 to 14.4 in JW663 for wild sugarcanes of the Honshu, and the average was 7.1. There was a negative correlation between the index of stem volume and juice brix for all wild sugarcanes at 1% level of probability (Figure 4).

Table 2. Juice brix and stem traits of Japanese wild sugarcanes in Exp. 2.

Category	Serial number	Collection site	Accession / Cultivar name	Juice brix (%)	Stem length (cm)	Stem diameter (cm)	Index of stem volume	Flowering
Wild sugarcane	1	Nansei Islands	JW90	7.4 ± 0.4	97 ± 5	0.73 ± 0.01	13.0 ± 0.9	Yes
	2		JW70	7.6 ± 0.7	102 ± 6	0.72 ± 0.01	13.3 ± 0.8	No
	3		Iriomote34	8.1 ± 0.3	128 ± 7	0.77 ± 0.01	19.0 ± 1.1	No
	4		JW294	8.7 ± 0.7	96 ± 6	0.57 ± 0.02	7.9 ± 1.1	No
	5		JW259	8.9 ± 0.5	127 ± 2	1.00 ± 0.02	32.1 ± 4.5	No
	6		99JW-18	9.5 ± 0.5	88 ± 10	0.67 ± 0.05	10.1 ± 2.5	No
	7		99JW-15	10.5 ± 1.0	66 ± 6	0.68 ± 0.02	7.8 ± 1.2	Yes
	8		99JW-10	10.8 ± 1.2	99 ± 3	0.75 ± 0.04	13.9 ± 1.7	No
	9		99JW-23	11.7 ± 1.5	95 ± 9	0.67 ± 0.06	11.1 ± 2.7	Yes
	10		JW223	14.8 ± 0.7	53 ± 4	0.50 ± 0.02	3.3 ± 0.5	No
		Average of Nansei Islands		9.8	95	0.71	13.1	
		Honshu	JW559	7.3 ± 0.8	116 ± 6	0.62 ± 0.02	11.3 ± 1.1	No
	12		JW529	10.1 ± 0.3	105 ± 13	0.62 ± 0.06	10.4 ± 3.1	No
	13		JW622	10.9 ± 1.8	121 ± 4	0.59 ± 0.02	10.5 ± 1.2	No
	14		JW663	11.7 ± 0.4	106 ± 8	0.73 ± 0.03	14.4 ± 2.5	No
	15		JW634	16.1 ± 0.8	63 ± 7	0.48 ± 0.03	3.7 ± 0.8	No
	16		JW545	16.7 ± 0.1	57 ± 6	0.48 ± 0.01	3.3 ± 0.5	No
	17		JW614	16.8 ± 0.6	59 ± 7	0.52 ± 0.00	3.9 ± 0.5	No
	18		JW577	17.1 ± 0.3	70 ± 8	0.53 ± 0.01	4.9 ± 0.7	No
	19		JW640	17.3 ± 0.7	53 ± 3	0.53 ± 0.01	3.7 ± 0.3	No
20	06JW-3		17.3 ± 1.0	83 ± 2	0.45 ± 0.02	4.3 ± 0.4	No	
	Average of Honshu		14.1	83	0.56	7.1		
Cultivar			NiF8	18.2 ± 0.8	182 ± 13	2.85 ± 0.19	368 ± 39	No
			KRFo93-1	15.4 ± 0.4	188 ± 7	2.13 ± 0.10	215 ± 27	No

Notes. The average value ± SE (n = 3) is given for each. NiF8 is a cultivar for sugar mill use and KRFo93-1 is a cultivar for forage use.

**Figure 3.** Comparison of juice brix measurements from Exp. 1 and Exp. 2.

Numbers beside the symbols indicate the serial number as given in Table 2. ** indicates a significant positive correlation at 1% level of probability.

4. Discussion

Ratoon crops from the genebank field without fertilizer were targeted in Exp. 1, while plant crops grown under conventional management were targeted in Exp. 2. Despite the differences in the experimental conditions, there was a positive correlation between the juice brix in

Exp. 1 and that in Exp. 2, and the juice brix values from both experiments were almost equal for the 20 tested wild sugarcanes (Figure 3). As for wild sugarcanes of the Nansei Islands, the average juice brix of 64 accessions was 8.6% in Exp. 1 (Table 1), which is consistent with Nagatomi (1985) who reported an average of 8.69% for 257 wild sugarcanes of the Nansei Islands. From these results, we judged that the brix value of the wild sugarcanes in this study had very good reproducibility.

The aim of this study was to identify the Japanese wild sugarcanes with high brix that could be used to improve the interspecific crossing of sugarcane. Nagatomi and Degi (2007) suggested that wild sugarcanes indigenous to the Honshu had high juice brix – this finding was our motivator to begin this study. Our results agreed with those of Nagatomi and Degi (2007); we were able to identify wild sugarcanes with high juice brix such as JW640 and 06JW-3, which were collected from the Honshu (Tables 1 and 2). Moreover, we could clarify that the median value of juice brix was significantly higher in the wild sugarcanes of the Honshu than in those of the Nansei Islands (Figure 2). Shimabuku et al. (1989) indicated that the brix of initial hybrids between commercial sugarcane and wild sugarcane was 12.91%, and it took two backcrosses to increase the brix of progeny clones to 16.27%. As shown by Shimabuku et al. (1989), nobilization usually requires a long-term effort, which is a significant problem for sugarcane breeders. The outstanding germplasms with high

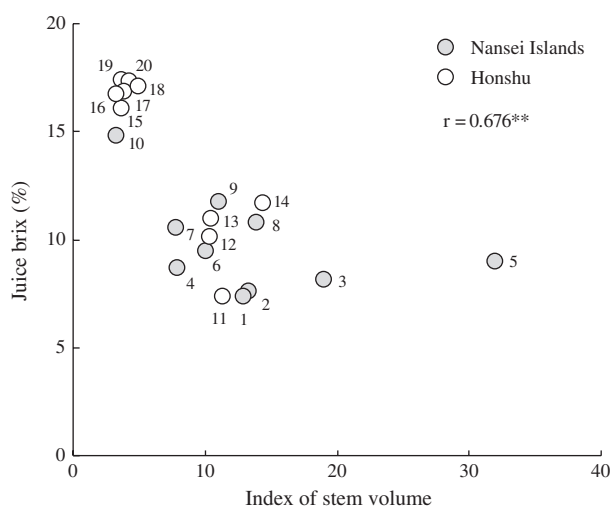


Figure 4. Relationship between the index of stem volume and juice brix in Exp. 2.

Numbers beside the symbols indicate the serial number as given in Table 2. **indicates a significant negative correlation at 1% level of probability.

juice brix identified in the present study are expected to overcome this obstacle of interspecific crossing and increase the efficiency of mobilization.

As for the quality of cane juice, Rao et al. (1973) indicated that the purity, which indicates the ratio of sucrose content to brix, was typically lower in wild sugarcanes than in *S. officinarum* and its hybrid clones. In this study, only brix was examined as an indicator of juice quality. Further research on the components of juice brix will be needed in the high-brix germplasms.

Interspecific hybrids with high biomass productivity can be obtained by crossing with wild sugarcanes (Nagarajan et al., 2000; Roach, 1977; Shimabuku et al., 1989). In previous work, we developed high-yielding cultivars for forage use through interspecific crossing with an overseas wild sugarcane, 'Glagah Kloet' (Sakaigaichi & Terajima, 2008; Sakaigaichi et al., 2014). In the current study, we found a negative correlation between the index of stem volume and juice brix (Figure 4), suggesting an important relationship between the two. Sugarcane breeders have tried to improve the productivity of their crop through introgression. Therefore, wild sugarcanes that located in the upper portion of scatter plot in Figure 4, such as 06JW-3, JW663, and JW259, might be given the high priority as breeding materials.

In conclusion, we identified outstanding wild sugarcanes based on juice brix. In the future, it will be necessary to confirm the characteristics of their progeny, such as juice brix and yield. This work is a critical step toward the improvement of the current interspecific breeding program.

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Disclosure statement

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