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Three descriptors for sugars to evaluate grape germplasm

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Summary

Content and composition of sugars of 259 grapevines including varieties, bred lines, rootstocks and wild species were determined. Wide variation existed in the sugar content. The sugar contents of varieties and bredlines were higher than those of rootstocks and wild species. The sugar components, fructose, glucose and sucrose were detected. Fructose and glucose were the primary sugars in approximately 1 : 1 ratio. Sucrose was present in a trace amounts, but two accessions of *Vitis rotundifolia* contained large amounts of sucrose. For the evaluation of the sugar composition, it was possible to discriminate clearly between high and low sucrose accessions by the ratio of glucose to fructose plus sucrose (α ratio).

Introduction

Sugars in grape are not only important in the alcoholic fermentation at the wine production, but also influence the taste of table grape (Amerine et al., 1979a). The great stress is, therefore, laid upon the documentation of sugars for evaluation of grape germplasm.

In an IBPGR description list for evaluation of grape germplasm, only the measurement of the value of soluble solids (° Brix value) in grape juice has been adopted for the documentation system of sugars (IBPGR, 1983). Sugars generally contribute a very large proportion to the ° Brix value in the ripening stage (Winkler et al., 1974a). However, the ° Brix value does not always run parallel with the sugar content since the ° Brix value represents the total value of soluble solids such as sugars, organic acids and others. Furthermore, it is impossible to determine an individual sugar content. As the ° Brix value alone is not sufficient to evaluate the content and composition of sugars, revision of documentation system for sugars should be required.

The objective of this study is to examine the variations of content and composition of sugars, and to propose a revised descriptor based on the variations of sugars in grape germplasm including varieties, bred lines, rootstocks and wild species.

Materials and methods

Plant material

In this study 259 grapevine accessions were used, 169 varieties, 81 bred lines, 5 rootstocks and 4 clones of 2 wild species (Table 1). These accessions were grown at the experimental vineyards of Fukuoka Horticultural Experiment Station, Fukuoka, Japan. The mature grapes were sampled from two or three clusters at the optimum ripening stage by visual observations in 1989.

Table 1. List of accessions used in this study

Populations	Parental species	Number of accessions
Varieties	*V. vinifera*	89
	V. labrusca × *V. vinifera*	78
	V. rotundifolia	2
Bred lines	*V. vinifera*	40
	V. labrusca × *V. vinifera*	41
Rootstocks	*V. vinifera* × *V. rupestris*	1
	V. vinifera × *V. berlandieri*	1
	V. berlandieri × *V. rupestris*	1
	V. doaniana	1
	V. rupestris × *V. candicans*	1
Wild species	*V. amurensis*	3
	V. coignetiae	1

Sample preparation

About 150 g of grapes were crushed by pressing, and then clarified by centrifugation at 5,000 g for 10 min. The supernatant juice sample was immediately frozen and stored at– 20° C in a sealed polypropylene tube until analysis.

Sugar content

Sugar content was determined by both ° Brix value and chemical analysis. The ° Brix value was measured with a handrefractometer using a two ml of juice sample. Chemical analysis was performed by a modified procedure based on Bertrand method (1906). The modified procedure was as follows: Two ml of juice sample was diluted to 100 ml with a deionized water. Ten ml of this solution was placed into a 100 ml conical flask. Ten ml of solution A (4% Copper (II) Sulfate, w/v) was added, and then 10 ml of solution B(20% Rochelle Salt, w/v and 15% Sodium Hydroxide, w/v) was added. The mixture was boiled for three minutes. After the supernatant liquid was discarded, and then the precipate (Cu_2O) was immediately dissolved with a 10 ml of solution C (5% iron (III) Sulfate, w/v and 10% Sulfuric Acid, v/v). This solution was titrated with solution D (0.5% Potassium Permanganate, w/v) until the color of solution changed from light blue to a purple red for 30 sec.

Sugar composition

Sugar composition was determined by a Tosoh HPLC equiped with a RI 8010 refractive index detector. Ten ml of juice sample was diluted to 25 ml with a deionized water and then filtered through a 0.45 μm filter. Injection volume was a 10 μl. The column (TSKgel Amide-80: 4.6 mm I.D. × 25 cm L) was operated at 80° C. Mobile phase was a mixture of acetonitrile and water (80 : 20), and flowed at 1.0 ml/min.

Results

Frequency of the ° Brix values in 259 accessions was normally distributed, ranging from 8.0 to 25.0% (Fig. 1a). The mean ° Brix values of varieties (17.1%) and bred lines (18.2%) were higher than those of rootstocks (10.9%) and wild species

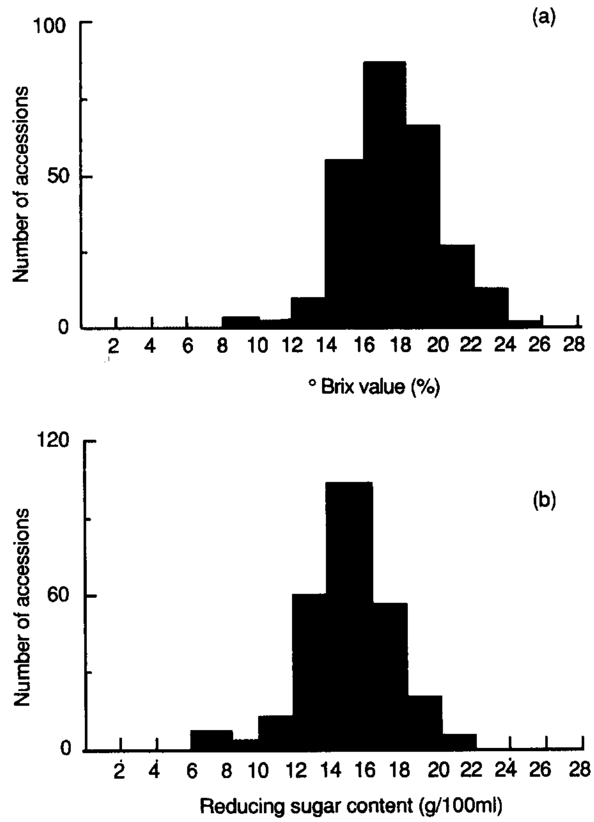

Fig. 1. Frequency distributions of the ° Brix value and reducing sugar content in grape juice of 259 accessions examined.

(13.4%), as shown in the upper column of Table 2. Frequency distribution of reducing sugar contents in the accessions ranged from 6.42 to 21.35 g/100 ml (Fig. 1b). The mean reducing sugar contents of varieties (14.92 g) and bred lines (15.65 g) were higher than those of rootstocks (6.42 g) and wild species (10.06 g), as shown in the middle column of Table 2.

HPLC profiles for sugar composition were obtained from 259 accessions examined with four major peaks designated as 1, 2, 3 and 4 in Fig. 2, each corresponding to H_2O, fructose, glucose and sucrose, respectively. The primary sugars were generally fructose and glucose, with trace amount of sucrose (Fig. 2a), but two accessions of *Vitis rotundifolia* contained a large amount of sucrose (Fig. 2b).

Frequency distributions of the relative content of fructose, glucose and sucrose in 259 accessions was illustrated in Figs 3a, 3b and 3c, respectively. The relative content of fructose ranged from 41.2 to 60.9%, while that of glucose was mostly the same (44.6 to 58.1%) except two accessions of *V. rotundifolia* whose relative contents of glucose were low as in 'Magnolia' (22.6%) and 'Wallace' (32.5%). The relative content of sucrose was generally below 2.0%, but those of two accessions of *V. rotundifolia* were high as in 'Magnolia' (36.2%) and 'Wallace' (14.8%).

The ratio of glucose to fructose (G/F ratio) has been used as an index for evaluation of sugar composition among accessions (Amerine & Thoukis, 1958). The G/F ratios of the accessions examined were ranged from 0.55 to 1.40, suggesting that the ratio seemed to be useful for evaluation of sugar composition among accessions. However, two high sucrose accessions of *V. rotundifolia* (G/F ratios were 0.55 and 0.61) could not be discriminated from the other low sucrose accessions by the G/F ratios (Fig. 4).

Linear relationships between glucose and fructose plus sucrose contents (G / [F + S] ratio) or between fructose and glucose plus sucrose contents ([G + S] / F ratio) in the all accessions were observed. However, as shown in Fig. 5, the two high sucrose accessions (HSA) could be more clearly dis-

Table 2. List of the proposed 3 descriptors for sugars in grape (° Brix value, reducing sugar content and α value) and frequency distributions of accessions examined for each descriptor

Descriptor	Grade	Code	Varieties	Bred lines	Rootstocks	Wild species
	below 12.0	1	0	0	4	1
° Brix value (%)	12.0 to 16.0	3	62	13	2	2
	16.0 to 20.0	5	90	52	0	1
	20.0 to 24.0	7	15	16	0	0
	exceed 24.0	9	0	1	0	0
Mean			17.1	18.2	10.9	13.4
	below 10.00	1	1	0	5	1
Reducing sugar content (g/100 ml)	10.00 to 14.00	3	59	14	0	3
	14.00 to 18.00	5	93	59	1	0
	18.00 to 22.00	7	14	9	0	0
	exceed 22.00	9	0	0	0	0
Mean			14.92	15.65	6.42	10.06
	below 0.20	1	0	0	0	0
α ratio*	0.20 to 0.40	2	1	0	0	0
	0.40 to 0.60	3	1	0	0	0
	0.60 to 0.80	4	3	0	0	0
	0.80 to 1.00	5	31	30	2	4
	1.00 to 1.20	6	126	52	2	0
	1.20 to 1.40	7	5	0	2	0
	1.40 to 1.60	8	0	0	0	0
	exceed 1.60	9	0	0	0	0
Mean			1.01	1.03	1.12	0.87

* : ratio of glucose to fructose plus sucrose (an index introduced in this study for evaluation of sugar composition in grape juice).

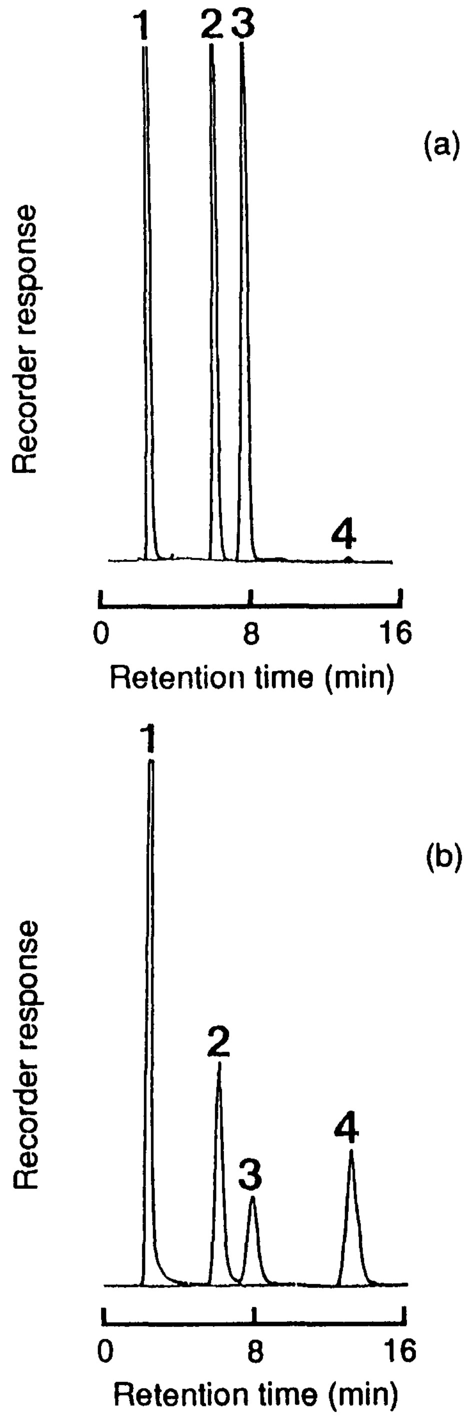

Fig. 2. Typical profiles of sugar composition by HPLC analysis (a) 'Ruby Cabernet' (*V. vinifera*) (b) 'Magnolia' (*V. rotundifolia*). Peak numbers on the chromatogram: 1 H_2O 2 Fructose 3 Glucose 4 Sucrose.

criminated from others in the former relationship (G / [F + S] ratio ranged from 0.29 to 1.39, HSA = 0.29 and 0.48) compared with the latter ([G + S] / F ratio ranged from 0.76 to 1.43, HSA = 0.90 and 1.43). Here, we designated the G/[F + S] ratio as the α ratio. According to the α ratio, as shown in the Table 2, it was possible to discriminate between high sucrose (below 0.60) and low sucrose accessions (exceed 0.60).

Discussion

The sugar content is generally expressed as the ° Brix value because of the highly positive correlation between the sugar content and the ° Brix value (r = 0.921: Lott & Barrett, 1967). As shown in Table 3, the mean ° Brix of cultivated species (*V. vinifera*: Lott & Barrett, 1967; Kliewer, 1970; *V. labrusca*: Caldwell, 1925; hybrids between *V. labrusca and V. vinifera*: Caldwell, 1925; Lott & Barrett, 1967) were generally high (17 to 20%), while that of *V. rotundifolia* (Carroll et al., 1971) and wild species (Nakagawa et al., 1980) were low (13% and 12%, respectively) except *V. coignetiae* (16%). In this study, the mean ° Brix of cultivated species were higher (varieties: 17%; bred lines: 18%) than that of rootstocks (11%) and wild species (13%), which is in agreement with previous reports, but it needs further research on wild species and rootstocks because of the lack of information.

A highly positive correlation was observed between the ° Brix value (X) and reducing sugar content (Y) in 259 accessions (Y = 0.827 + 0.815X, r = 0.859). If the objective is a rough estimation of sugar content, measurement of the ° Brix value is sufficient. However, the ° Brix value can not always be regarded as an index of the sugar content since the effect of temperature is not negligible (Amerine et al., 1979b). Furthermore, high organic acid accessions must have a correspondingly higher ° Brix value if the sugar content is to be at a similar level (Lott & Barrett, 1967). Generally fructose and glucose were the primary sugars and sucose was present in trace amounts from the HPLC analysis, indicating that the measurement of reducing sugars would be a regular analysis as the total sugar content of grape.

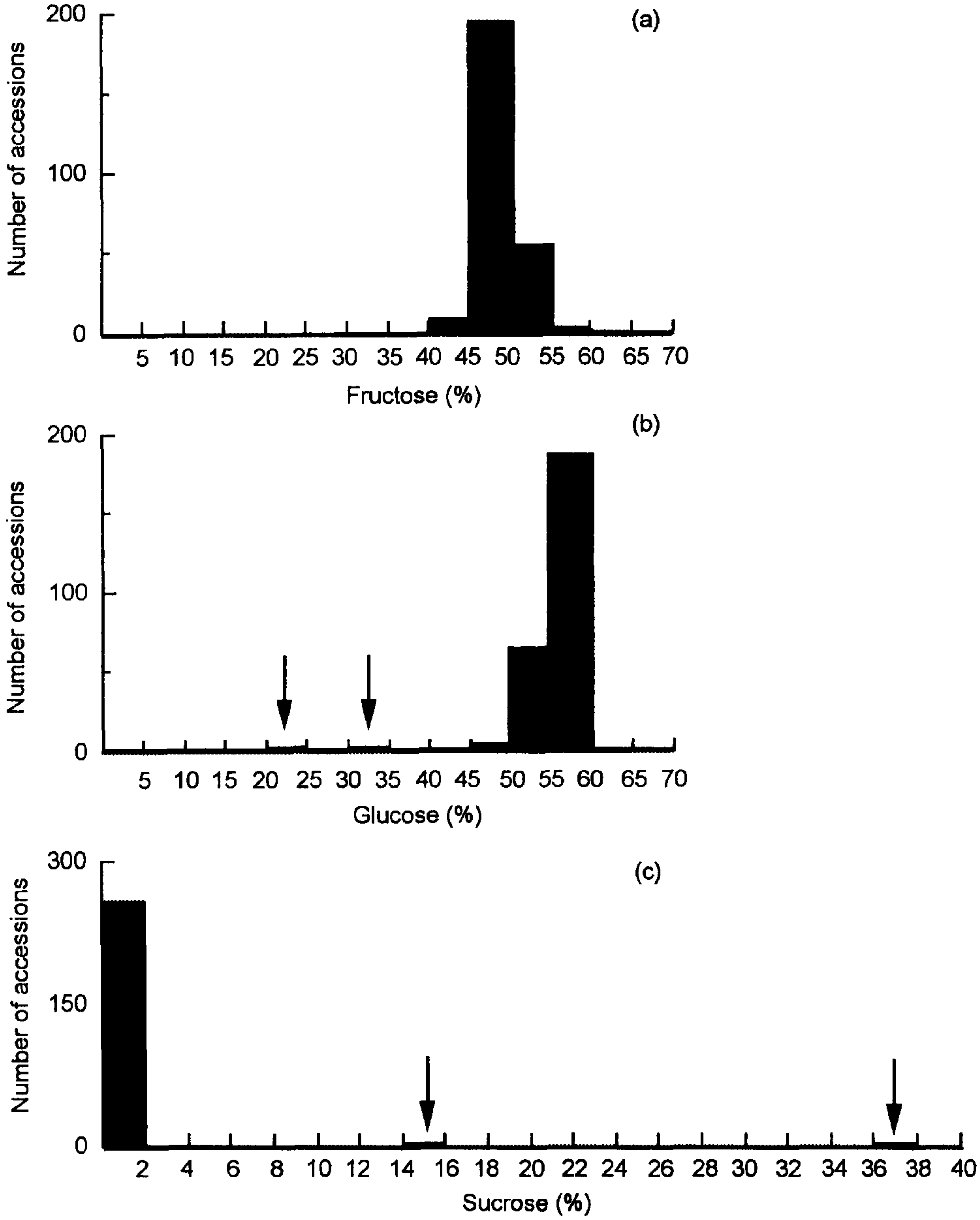

Fig. 3. Frequency distributions of relative contents of fructose, glucose and sucrose in grape juice of 259 accessions. Arrows indicate accessions of *V. rotundifolia* with high sucrose contents.

It is, therefore, desirable to measure the reducing sugar content simultaneously with the ° Brix value evaluating sugar content in grape germplasm.

Fructose and glucose were approximately present in 1 : 1 ratio, and sucrose was present in trace amounts except in *V. rotundifolia* (Fig. 3). These findings are in agreement with previous investigations (Kliewer, 1966; Kliewer, 1967; Lott & Barrett, 1967; Carroll et al., 1971; Notsuka & Shiraishi, 1982).

The glucose to fructose ratio (G/F ratio) is a use-

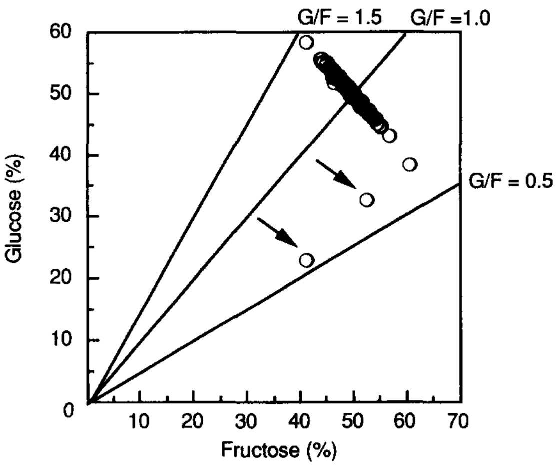

Fig. 4. Relationship between fructose and glucose contents in 259 accessions, in which arrows indicate accessions of *V. rotundifolia* with high sucrose contents. G/F: Ratio of glucose to fructose.

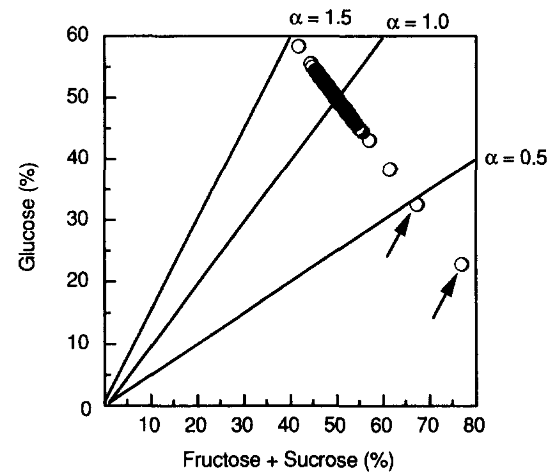

Fig. 5. Relationship between fructose plus sucrose and glucose contents in 259 accessions, in which arrows indicate accessions of *V. rotundifolia* with high sucrose contents. α: Ratio of glucose to fructose plus sucrose.

ful index for evaluation of sugar composition. The G/F ratios of high fructose accessions generally ranged from 0.70 to 1.00, while those of high glucose ones were 1.00 to 1.20 (Gallander, 1974). Previous investigations of high sucrose accessions are reviewed in Table 4. The sucrose content of 8 cultivars in hybrids between *V. labrusca* and *V. vinifera* ranged from 15.22 ('Bath') to 32.56% ('Sweet Blue'), and the G/F ratio of these cultivars varied from 0.58 to 0.83 (Lott & Barrett, 1967). Carroll et al. (1971) reported that the sucrose content and G/F ratio of *V. rotundifolia* ranged from 0.63 ('Scuppernong') to 32.72% ('Hunt') and 0.90 to 1.04, respectively.

These facts indicates that the G/F ratio is insufficient for discriminating between high sucrose and low sucrose accessions. Therefore, the oridinary index evaluating sugar composition (G/F ratio) should be revised considering the variation of sucrose in addition to that of fructose and glucose.

In terms of modificiation of the G/F ratio, two ratios of glucose to fructose plus sucrose (G / [F + S] = α) and glucose plus sucrose to fructose ([G + S] / F) were compared. The former (α ratio) ranged from 0.29 to 1.39 and discriminated clearly between high sucrose (0.29 and 0.48) and low sucrose accessions (more than 0.64) as shown in Fig. 5, while the latter ranged from 0.76 to 1.43 and could not discriminated between high sucrose (0.90 and 1.43) and low su-

Table 3. Reference reviews of the range and mean of the ° Brix value in *Vitis*

Parental species	Range (%)	Mean (%)	Authors
Cultivated species			
V. vinifera	15.4 to 21.6	18.9	Lott & Barrett, 1967
V. vinifera	16.8 to 24.0	19.8	Kliewer, 1970
V. labrusca	13.0 to 21.0	16.8	Caldwell, 1925
V. labrusca × *V. vinifera*	15.0 to 21.9	18.6	Caldwell, 1925
V. labrusca × *V. vinifera*	15.6 to 22.2	18.2	Lott & Barrett, 1967
V. rotundifolia	10.0 to 18.0	13.2	Carroll et al., 1971
Wild species			
V. flexuosa	11.5 to 13.0	12.3	
V. ficifolia	9.4 to 19.5	12.3	Nakagawa et al., 1980
V. amurensis	10.4 to 15.6	12.1	
V. coignetiae	13.2 to 18.8	16.2	

Table 4. Reference reviews of sugar composition in several cultivars having more than 10 per cent of sucrose

Cultivars	Per cent of:			G/F ratio*	α ratio**	Autors
	Fru.	Glu.	Suc.			
Bath	50.11	34.67	15.22	0.69	0.53	
Buffalo	42.34	35.85	21.81	0.85	0.56	
Captivator	41.03	33.23	25.74	0.81	0.50	
Champagne	45.11	26.99	27.90	0.60	0.37	Lott & Barrett, 1967
Erie	38.36	31.70	29.94	0.83	0.46	
Fredonia	46.52	34.73	18.75	0.75	0.53	
Kendaia	38.57	29.25	32.18	0.76	0.41	
Sweet Blue	42.74	24.70	32.56	0.58	0.33	
Mean	43.10	31.39	25.51	0.73	0.46	
Albemarle	38.98	37.37	23.65	0.96	0.60	
Dearing	35.53	39.90	22.17	1.02	0.65	
Hunt	33.33	33.95	32.72	1.04	0.51	
Magoon	43.38	38.16	18.46	0.90	0.62	Carroll et al., 1971
Magnolia	37.95	39.14	22.91	0.98	0.64	
Thomas	43.49	42.59	13.92	0.98	0.74	
Tropsail	38.11	37.83	24.06	0.99	0.61	
Mean	38.68	38.33	22.56	0.98	0.62	

* : the ratio of glucose to fructose.
** : the ratio of glucose to fructose + sucrose.

crose accessions. The α ratio of high sucrose accessions previously reported ranged from 0.33 to 0.74 and the mean was 0.54 (Table 4). Considering previous and our finding, the accession expressed below 0.60 can be expected to be high in sucrose. It is considered that the α ratio is greater than the [G + S] / F ratio for evaluating sugar composition since the latter ratio assess the variation of sucrose itself as that of glucose.

In the IBPGR documentation system of sugars (IBPGR, 1983), the ° Brix value is recorded as discontinuous 5 classes (1: < 13%, 3: 15–16%, 5: 18–19%, 7: 21–22%, 9: > 24%) rather than a continuous variation, as generally believed. Furthermore, no research publications have reviewed the basic concept for the documentation of sugars in grape germplasm. Therefore, the basic concept for the documentation of sugars in grape germplasm was discussed in terms of content and composition of sugars.

The optimum range of eating quality (palatability) in table grape is between 16 and 21% though the acidity affects markedly the palatability (Winkler, 1932). In contrast to table grape, the optimum maturity of wine grape occurs between 20 and 25% (Winkler et al., 1974b), but the fermentation rate is retarded if the ° Brix value exceeds 25% (Amerine et al., 1979b). The degree of sweetness differs according to the kinds of sugars (Fructose = 150; Glucose = 70; Sucrose = 100, sucrose taken as 100: Louis, 1955). Accession of low α ratio (below 1.00) from similar levels of the ° Brix values should be selected for producting sweet table grape and wine. Conversely, accession expressed high α value (exceed 1.00) at the full- or over-ripeness can be of a great acceptance for midseason and late marketing since they will be less senescent due to less glucose breakdown (Kliewer, 1965; Notsuka & Shiraishi, 1982).

Evaluation of sugars in grape germplasm is normally performed in a one place for several years because of the large plant size and cost. The mean standard deviations (MSDs) of the ° Brix values and the α ratios were 1.52 and 0.09 of 12 cultivars for 3 years (Carroll et al., 1971), and were 1.26 and 0.06 of 8 cultivars for 6 years (Rice, 1974), respectively. The mean coefficient of variations (MCVs) of the ° Brix values and the α ratios were 9.60 and 6.88%

(Carroll et al., 1971) and were 7.88 and 6.33% (Rice, 1974), respectively. These facts indicate that the class interval (CI) of the sugar content needs to be larger than that of sugar composition. Therefore, we defined the CI of descriptor for sugar content and sugar composition as 4.0 and 0.2 based on the range of ± 2MSDs.

In conclusion, as shown in Table 2, we proposed three descriptors for sugars of grape germplasm according to the descriptor state of IBPGR as follows: ° Brix value (9 classes: 1 = below 12.0%; 9 = exceed 24%, CI = 4.0%), reducing sugar content (9 classes: 1 = below 10.00 g; 9 = exceed 22.00 g, CI = 4.0 g) and α ratio (9 classes; 1 = below 0.20; 9 = exceed 1.60, CI = 0.20).

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