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A Preliminary Study on Cross Breeding of Acid Citrus with Reference to Juice Qualities and Physical Fruit Characteristics

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Fruits of F-l progenies of high-acid citrus and their parents were evaluated in 1992, 1993, and 1994 for their juice quality and physical fruit characteristics. After statistical adjustment for yearly variation, the results show that most progenies possessed high acidity but, the fruits were much smaller and lighter than their large or heavy fruit parents. Based on the degree of color development, early or late maturity seemed to be inherited. Peel weight of the progenies was widely distributed toward as light as the lightest, parents and as heavy as the heaviest ones. The juice weight was similarly distributed as peel weight. Fruit shape was skew-distributed toward globose or oblate.

INTRODUCTION

Quantitave genetic analysis has been a major method of interest in studying inheritances of traits in many horticultural crops. From the analysis, heritability and the nature of genetic variances of many traits as genetic basis in selecting promising parents and progenies for breeding programs are known in vegetables such as cucumber (McCreight et al., 1978) and tomato (Wessel-Beaver and Scott, 1992), fruits such as sweet cherry (Hansche *et al.,* 1966 and 1975), apple (Klein, 1958), avocado (Lavi et *al.,* 1991 and 1993), blueberry (Finn and Luby, 1992), peach (Hansche et al., 1972a), and nuts such as almond (Kester *et al.,* 1977), walnut (Hansche *et al.,* 1972b), and pecan (Thompson and Baker, 1993). A method of study in Mendelian genetics may also result in significant contributions for breeding programs such as used in apple (Kimball, 1930; Bishop, 1961; Nybom, 1959). However, the genetic basis and mode of inheritance of most fruit quality traits in citrus are still unknown although planned and documented studies of breeding and genetic have been recorded since 1893 (Cameron and Frost,, 1968).

A low acidity imparted by a low-acid pummelo *(Citrus grandis* L. Osbeck) has received a great deal of interest in developing 'table' citrus having high soluble solids-acid ratios (Soost and Cameron, 1961; Cameron and Soost, 1974, 1977, and 1979). In Japan, as an easy-peeled, good-looking 'table' citrus is preferred, the inheritances of peeling (Yamamoto et **al.,** 1988) and peel texture (Yamamoto *et al.,* 1990) were studied. Until reports on the heritability and the nature of genetic variances of citrus traits are available, citrus breeders depend mostly only on some known-inheritance of limited citrus species in selecting parents and progenies.

Comparing with 'table' citrus, acid citrus is less treated by any breeding program. An acid citrus breeding program was recorded in 1924, but the program was soon terminated (Cameron and Frost, 1968). Since then, no hybrids of acid citrus have been developed via sexual hybridization. Efforts to develop hybrids of acid citrus have been on record in Japan (Koike, 1993; Koike and Yamao, 1992). However, their data are extremely limited to give a thourough picture of the genetic aspect in acid citrus breeding.

Unlike 'table' citrus, high acidity is preferred in acid citrus. Together with juice percentage, it determines the juice quality demanded by juice processors. Besides, physical fruit characteristics are also important. Fruit size and fruit weight determine the number of fruit per carton. Fruit shape is also an important factor as slices of acid citrus are often served in salads and other dishes; an ellipsoid shape may give more slices than globose or oblate. With the existing condition of mostly unknown inheritance of fruit quality traits, breeding studies in acid citrus are left further behind and breeders are left with few clues for selecting promising parents and progenies, especially for juice quality traits.

The present paper reports juice qualities and physical fruit characteristics of F-l progenies from crosses of several acid citrus evaluated in 1992, 1993, and 1994. These results may provide some guidelines for others who are carrying on acid citrus breeding programs.

MATERIALS AND METHODS

The materials used in this study were sampled from progenies grown at the Fruit Trees Experiment Station of Kyushu University in November 16, 1992, 1993, and 1994. The progenies were budded on Satsuma mandarin (Citrus unshiu Marc.). The parents crossed were Hanayu (C. *hanayu* Hort. ex Shirai), Kabosu (C. *sphaerocarpa* Hort. ex Tanaka), Sudachi (C. *sudachi* Hort. ex Shirai), Yuzu (C. junos Sieb. ex Tanaka), Daidai *(C. aurantium* Linn. var Cyanthifera Y. Tanaka), 'Lisbon' lemon (C. *limon* Burm. f. Lisbon), 'Kusaie' lime (C. *limonia* Osbeck f. Kusaie), 'Ethrog' citron (C. *medica* var Ethrog Engl.) and Chinotto 'citrange' (a description given by Hodgson, 1967, indicates that our Chinotto seems to be a dwarf Chinotto of myrtle-leaf orange, C. *myrtifolia* Rafinesque, not a citrange). With a relatively high sugar content and a low acidity, the Chinotto is not an acid citrus but as it produced progenies with a high acid level it is included in this study. In all parents and progenies, when the number of fruits exceeded 10 fruits, 10 fruits were sampled randomly around the canopy. Otherwise, all available fruits were used. The fruits were peeled, the juice was extracted with a hand-pressed juicer and then centrifuged at 2,500 rpm for 20 minutes. A hundred ml of the supernatant juice was used for analyses. Fruit, peel, and juice of the samples were weighed individually. Fruit size as a fruit diameter plus fruit height was developed as suggested by Lentz (1967).

Total sugar content was determined as follows: 4 ml of deionized water was added to 1 ml juice, and then 5 ml of 4% H_2SO_4 was added into it. After boiling for 30 minutes, this solution was neutralized with 2 N NaOH. This solution was then treated as in the reducing sugar determination of the modified procedure of Bertrand (Shiraishi, 1993). Total acid content was determined in 1 ml juice by titration with 0.1 N NaOH and phenolphthalein as indicator after passage through a column (1.6 cm I. D. $\times$ 4 cm) of cation-exchange resin Amberlite IR-120.

Yearly differences are known to add considerably to the variability of characters in many plants (Hansche et al., 1966, 1972a and 1972b; Shiraishi, 1995; Thompson and Baker, 1993). To minimize this source of error, least-square estimates of year effects were obtained and all measurements were adjusted accordingly. The statistical procedures for obtaining the estimates are those applicable to non-orthogonal experiments with unequal number of observation within cells and with missing cells. The use of these methods has been discussed and described in detail by Henderson (1953) and later by Searle and Henderson (1961).

The appropriate statistical model from which available data provided least squares estimates of year effects, $Y_{ijk} = \mu + g_i + y_j + (gy)_{ijk}; i = 1, 2, \ldots q, j = 1, 2, r$, and $k = 0, 1, s$, has been described elsewhere (Hansche *et al.*, 1966 and Hansche *et al.*, 1972a). The number of genotype, q, was a total of 102, consisting of 9 parents and 93 progenies, and the number of year, r, was 3. The total number of observations over genotypes and years varied from traits to traits between 414 and 866. The number of observations of each genotype in each year, s, was either 0 or 3 in sugar and acid measurements, and as low as 0 and as high as 10 in physical fruit measurements. The linear equations for each trait were then solved for $\hat{y}_j$'s and the data were adjusted accordingly; *i.* e., $\hat{Y}_{ijk} = Y_{ijk} - \hat{y}_j$.

RESULTS AND DISCUSSION

The performances of progenies were markedly affected by yearly differences (Table 1). These annual variations complicate the discrimination of progeny differences when comparisons are made on progeny's performances in different years. The statistical removal of these effects would be expected to increase the ability to discriminate the progenies and simplify the analysis.

Juice qualities and physical fruit characteristics of the parents used are shown in Table 2. The acid citrus used had a very high acidity, higher than obtained by Yamaki (1988 and 1989), but the acidity of Yuzu agreed with Sawamura et *al.* (1986). A wide difference in total sugar content was present. During maturation, an increase in sugar content might have occured while acidity was decreasing. At harvest, Chinotto and Daidai were still green and Lisbon was yellowish green, while other parents had been in a yellow

Table 1. Least square estimates of year effect for fruit, qualities[y].

Fruit qualities	1992	1993
Total acid content	-11.60	-3.91
Total sugar content	-7.78	-4.38
Fruit size[x]	0.18	0.08
Fruit weight	12.38	4.05
Peel weight	5.46	4.58
Juice weight	3.88	0.14
Fruit shape index[z]	-0.05	-0.01

[y]Least square estimate of year effect in 1994 was set to zero;
[x]Fruit diameter plus fruit height; [z]Fruit diameter-to-fruit height ratio

Table 2. Juice qualities and physical fruit characteristics of the parents[u].

	TA[y]	TS[y]	Fruit color[x]	Fruit size[w] (cm)	Fruit weight (g/fruit)	Peel weight[v] (%)	Juice weight[v] (%)	FSI[z]
	----(mg/ml)----							
Chinotto	18.63	58.39	Green	8.23	36.73	29.10	31.53	1.09
Sudachi	60.94	44.18	Yellow	7.65	31.39	25.29	33.35	1.24
Kabosu	55.50	36.04	Yellow	12.49	136.76	32.98	34.73	1.20
Daidai	54.63	35.16	Green	16.17	224.70	40.65	33.24	1.09
Yuzu	61.91	25.54	Yellow	12.16	126.54	39.12	19.84	1.27
Hanayu	67.07	24.62	Yellow	9.44	45.88	35.29	25.78	1.11
Lisbon	74.30	16.89	yGreen	15.84	110.93	37.16	29.00	0.77
Citron	71.19	18.32	Yellow	17.11	256.35	48.11	17.77	0.71
Kusaie	74.92	12.42	Yellow	7.37	25.43	24.26	39.83	0.98

[u] Data after year-effect adjustment; [y] TA and TS are total acid and total suger contents, respectively; [x] Fruit color in November 16, 1992, 1993, and 1994. Yellow includes its advanced color such as yellowish orange; [w] Fruit. diameter plus fruit height.; [v] As % of fruit wt. data after year effect adjustment; [z] Fruit shape index, a fruit diameter-to-fruit height ratio.

stage. The parents were clearly distinguisable on their physical fruit characteristics. The fruit size of Citron, Lisbon, and Daidai was the biggest, Kabosu and Yuzu were medium, and Chinotto, Sudachi, Hanayu, and Kusaie were the smallest. The heaviest fruits were those of Daidai and ‘Ethrog’ citron, but they had the thickest peel. Kusaie was the smallest with the thinnest peel but contained the highest percentage of juice. The citron had the least juice content, almost similar to the juice of Yuzu. A study on fruit qualities of lemon from various countries of origin conducted by McDonald and Hillebrand (1980) showed that the largest fruit had the thickest peel together with a lower percentage of juice. The shape of Lisbon and citron was ellipsoid, Kusaie was globose, Daidai and Chinotto were less oblate than Sudachi, Kabosu, and Yuzu.

Frequency distribution of total acid and total sugar contents of the progenies are shown in Table 3. Of total progenies examined, 70.27% had an acidity as high as their parents, and 79.57% had a sugar content ranged between 11 and 50 mg/ml, which was also the range of sugar content of the parents. It should be noted, however, that the progenies were harvested at the same time so that some progenies might have been overriped. Of total progenies, 63.44% was in a yellow stage (Table 4). It means that the actual number of progenies of high acidity could be much higher than the presented data. Soost and Cameron (1961) reported that a low-acid pummelo crossed with several mandarin and orange varieties produced hybrids of moderate acidity, while acid pummelos produced hybrids of high acidity. In apple, sweet flavor was known to be determined by one, commonly occuring, recessive gene (Nybom, 1959).

As shown previously in Table 2, the fruits of Chinotto, Daidai, and Lisbon were not as mature as those of other acid citrus investigated on the time of harvest. The fruits of Kabosu, Hanayu, Yuzu, and Sudachi are known to develop yellow color around October, earlier than the fruit of Lisbon of the same age, and much earlier than Daidai. These differences seemed to be inherited to the progenies (Table 4). Kabosu, Hanayu, Yuzu, and

Table 3. Frequency distribution of total acid and total suger contents among the progenies.

Seed parent	Pollen parent	Total acid content, (mg/ml)				Total sugar content (mg/ml)			
		430	31-50	51-70	271	110	11-30	31-50	≥51
Daidai	Lisbon		1	1	2	2	1	1	
Lisbon	Daidai			1	1		1	1	-
	Kabosu		2	7	-		5	3	1
	Sudachi	-	1	1	2	1	2	1	
	Yuzu			3	3	1	4	1	-
Hanayu	Chinotto	-	2	2	-		2	1	
	Kabosu	1	6	7	3	1	6	6	4
	Lisbon			4	5	5	4	-	
	Sudachi		1	2	1		2	2	
	Yuzu		1	4	3	1	3	3	1
Sudachi	Kabosu		2	3				4	1
	Lisbon		1						1
	Yuzu		3	2	-	-	1	4	
Yuzu	Citron			1			1		
	Daidai		1	-	-			1	-
	Kabosu			2	-		1	1	
	Kusaie			7	1		6	2	-
	Lisbon			2	-		1	1	
	Sudachi		1	1	:	-	1	1	-

Table 4. Frequency distribution of fruit color among the progenies.

Seed parent	Pollen parent	Fruit color[z]			
		Green	yGreen	gYellow	Yellow
Daidai	Lisbon	3	1		
Lisbon	Daidai	1	1		
	Kabosu	1	4		4
	Sudachi		1		3
	Yuzu				6
Hanayu	Chinotto	-		2	1
	Kabosu		1	3	13
	Lisbon	2	4	1	2
	Sudachi			1	3
	Yuzu	1		1	6
Sudachi	Kabosu				5
	Lisbon				
	Yuzu			1	4
Yuzu	Citron				1
	Daidai		1		
	Kabosu				
	Kusaie		2	1	5
	Lisbon				2
	Sudachi	1			1

[z] yGreen=yellowish green; gYellow=greenish yellow; yellow includes its advanced color such as yellowish orange.

Table 5. Frequency distribution of fruit size among the progenies.

Seed parent	Pollen parent	Fruit size (cm)[z]				
		≤6	7-10	11-14	15-18	219
Crossings among parents of big and/or medium size fruits:						
Daidai	Lisbon		2	1	1	
Lisbon	Daidai					1
	Kabosu		3	5	1	
	Yuzu		1	4	1	
Yuzu	Citron			1		
	Daidai		1			
	Kabosu		2			
	Lisbon		2			
		(0)	(12)	(11)	(3)	(1)
Crossings with parents of small size fruits:						
Lisbon	Sudachi		1	3		
Hanayu	Chinotto		3			
	Kabosu		14	3		-
	Lisbon		6	3		
	Sudachi		4			
	Yuzu		8			
Sudachi	Kabosu		4			
	Lisbon		1			
	Yuzu		4			
Yuzu	Kusaie		8			
	Sudachi			2		
		(1)	(53)	(12)	(0)	(0)

[z] Fruit diameter plus fruit height. Numbers in parentheses are total frequency distributions.

Sudachi seemed to transfer a factor of early maturity to their progenies. Of 93 progenies, 63.44% had developed yellow color and its advanced color, such as yellowish orange. Daidai and Lisbon, on the other hand, seemed to transfer late maturity to their progenies. High heritability and additive genetic variation of maturity were found in sweet cherry (Hansche et al., 1966) and peach (Hansche et al., 1972a).

Fruit size was skew-distributed toward smaller size (Table 5). Parents having medium and big size fruits seemed to carry also a factor controlling a small size. They produced 44.44% of progenies with small fruits. Crossing parents having small fruits such as Sudachi, Hanayu, Chinotto, and Kusaie with parents having medium or big fruits produced much more progenies having fruit size as small as the small fruit parents. The

Table 6. Frequency distribution of fruit weight among the progenies.

Seed parent	Pollen parent	Fruit weight (g/ fruit)[z]						
		≤20	21-50	51-80	81-110	111-140	141-170	1171
Crossings among parents of heavy and/or medium fruits:								
Daidai	Lisbon			2	1			
Lisbon	Daidai		1					
	Kabosu		1	4	2	1	1	
	Yuzu		1	1	2		1	
Yuzu	Citron						1	
	Daidai			1				
	Kabosu		1	1				
	Lisbon		1	1				
		(0)	(5)	(10)	(5)	(1)	(3)	(3)
Crossings with parents of light fruits:								
Lisbon	Sudachi			3	1			
Hanayu	Chinotto		2	1				
	Kabosu	1	10	5	1			
	Lisbon	1	4	3	1			
	Sudachi	1	2	1				
	Yuzu	1	4	3				
Sudachi	Kabosu		3	2				
	Lisbon			1				
	Yuzu		2	3				
Yuzu	Kusaie		8					
	Sudachi				1	1		
		(4)	(35)	(22)	(4)	(1)	(0)	(0)

[z] Numbers in parantheses are total frequency distributions.

same tendency was found in fruit weight (Table 6). Crossings among parents having fruits of heavy or medium weight produced more progenies with much lighter fruits. When a progeny with light fruit was used as a parent, the progenies were distributed toward the light fruit parent. In apple, factors which determine small size appear to be dominant to those which tend to produce large size (Klein, 1958; Bishop, 1961).

The fruit shape index was skew-distributed toward globose and oblate (Table 7). Only 21.05% of the total progenies of Lisbon and citron had the fruit shape of Lisbon or citron. No ellipsoid progeny was produced from crossings among globose and/or oblate parents. Klein (1958) found that the tendency toward roundness or oblateness was dominant to the tendency toward length.

Table 7. Frequency distribution of fruit shape index among the progenies.

Seed parent	Pollen parent	Fruit shape index[z]		
		Ellipsoid (≤0.8)	Globose (0.9-1.1)	Oblate (≥1.2)
Crossings with an ellipsoid parent:				
Daidai	Lisbon		4	
Lisbon	Daidai	2		
	Kabosu	3	6	
	Sudachi		4	
	Yuzu	2	4	
Hanayu	Lisbon	1	5	3
Sudachi	Lisbon		1	
Yuzu	Citron		1	
	Lisbon		1	
		(8)	(26)	(4)
Crossings among globose and/or oblate parents:				
Hanayu	Chinotto		2	
	Kabosu		11	6
	Sudachi		2	2
	Yuzu		4	4
Sudachi	Kabosu		4	
	Yuzu		3	2
Yuzu	Daidai			
	Kabosu			2
	Kusaie		6	2
	Sudachi			2
		(0)	(32)	(23)

[z] Fruit diameter-to-fruit height ratio. Numbers in parantheses are total frequency distributions

Peel weight was distributed to as light as the peel of Kusaie or Sudachi and as heavy as that of citron and Daidai (Table 8). Regardless of the parents crossed, both progenies having lighter and heavier peel than the parents were produced. A similar distribution was also noted on juice weight (Table 8). The International Standardisation for citrus fruit requires a minimum juice content of 25% for acid fruit other than lemons (OECD, 1971). Based on this standard value, 84.95% of the progenies passed the minimum standard.

A great heterozygous nature and a quantitative inheritance of most characters are known in citrus (Cameron and Frost, 1968). These natures were observed in all traits we examined. These indicate that a wide genetic resource is present in acid citrus. Factors controlling high acidity seemed to be less complex than those controlling physical fruit characters. A high acidity imparted by acid pummelos to their progenies was reported by

Table 8. Frequency distribution of peel and juice weights[z] among the progenies.

Seed parent	Pollen parent	Peel wt. (%)				Juice wt.(%)			
		≤29	30-35	36-41	≥42	119	20-28	29-37	≥38
Daidai	Lisbon	1		1	2		3		1
Lisbon	Daidai				1		2		
	Kabosu	4	2		3	3	4	2	-
	Sudachi	3			1	1	1		2
	Yuzu	2			3	1	2	3	-
Hanayu	Chinotto	2		1	-		1	2	-
	Kabosu	7	5	2	3	2	8	5	2
	Lisbon	5	2	2	-	2	4	2	1
	Sudachi	3		1	-			4	
	Yuzu	2	1	3	2	3	5		
Sudachi	Kabosu	4	1	-	-	-	-	1	4
	Lisbon		1					1	-
	Yuzu	3	1	1	-	1	-	2	2
Yuzu	Citron		1				1		
	Daidai				1		1		
	Kabosu					1	-	1	
	Kusaie	7		1	-		4	2	2
	Lisbon		1				1	1	-
	Sudachi		1				2		

[z] As % of fruit weight, data calculated after year effect adjustment.

Soost and Cameron (1961). A dominant nature of acidity over sweetness was reported in apple (Klein, 1958; Nybom, 1959). As shown in Table 3, more progenies of high acidity than the presented data were possible if they were harvested at their prime maturity.

As shown in Table 5 and 6, more progenies of smaller and lighter fruits were produced. The dominant inheritance of small fruits over large fruits were reported in apple (Klein, 1958; Bishop, 1961). In avocado, due to low additive and non-additive genetic variance of fruit weight, screening among selfing progeny was advised only when decrease of fruit weight is the breeding objective (Lavi et al., 1991). The data presented in Table 5 and 6 suggest that crossing parents having small fruits such as Sudachi, Hanayu, Chinotto, and Kusaie with parents having larger fruits may result in some progenies having larger fruits than the small parents. However, choosing parents based on this character can not be done with certainty due to unavailable information on citrus and variable results found in different plants. High heritability of this character was found in walnut and peach (Hansche et *al.,* 1972a and 1972b) but low to moderate in pecan (Thompson and Baker, 1993), and low in avocado (Lavi et al., 1991 and 1993).

Another significant results suggested from this initial evaluation are early maturity of Hanayu, Sudachi, and Yuzu, and late maturity of Daidai and Lisbon, indicating that a wide genetic variation of maturity is present in acid citrus. Daidai, however, possesses some unfavourable characters such as granulation and a tremendous decrease of acidity during maturation (Yamada and Nishiura, 1977).

Differences in fragrance are of interest in acid citrus breeding programs dealing with Japanese acid citrus because they have a unique fragrance with different compositions of peel oils (Yang et al., 1992). This may be the field that should receive more attention in acid citrus breeding programs.

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