

# Repeatability, Optimal Sample Size of Measurement and Phenotypic Correlations of Quantitative Traits in Guava

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## ABSTRACT

Five fruits from each of 11 guava genotypes were evaluated in dry and early rainy seasons under Thailand conditions for fruit weight, flesh thickness, flesh weight, seed cavity (central pulp) weight, fruit firmness, total soluble solids, titratable acidity, juice acidity, and ascorbic acid to estimate repeatability (R), phenotypic correlations (r), and to predict the optimal sample size. The repeatability of the fruit weight, flesh thickness, flesh weight, seed cavity weight, titratable acidity, juice acidity, and ascorbic acid were relatively high ( $R \geq 0.60$ ). The flesh thickness, titratable acidity, juice acidity, and ascorbic acid were the traits with highest estimates, 0.85, 0.85, 0.87, 0.76 and 0.85, 0.83, 0.84, 0.80 in dry and early rainy seasons, respectively. Based on a threshold of 10% increase in relative efficiency, a sample of three fruits was sufficient for evaluating guava fruit traits. Most physical traits (fruit weight, flesh thickness, flesh weight, and seed cavity weight) had weak negative correlations ( $-0.25 \leq r \leq -0.38$ ) with chemical traits (total soluble solids, titratable acidity, and ascorbic acid). Fruit firmness had no correlation with all other fruit traits. There were strong positive correlations between fruit weight and flesh thickness ( $r = 0.81$ ), flesh weight ( $r = 0.99$ ), and seed cavity weight ( $r = 0.88$ ). Therefore, fruit weight could be used as an indirect selection for flesh thickness, flesh weight, and seed cavity weight.

**Key words:** *Psidium guajava* L., breeding, quantitative trait analysis, fruit qualities

## INTRODUCTION

Guava (*Psidium guajava* L.) is native to tropical America and presently found distributing in several tropical and subtropical regions (Cobley, 1976) such as India, South Africa, Brazil, Cuba, Venezuela, New Zealand, the Philippines, Hawaii, Florida, and California (Yadava, 1996), Vietnam (Le *et al.*, 1998), and Thailand (Tate, 2000). In part because it is a highly variable species for many morphological and horticultural traits, tolerant to environmental stress such as salinity (Nakasone and Paull, 1998), and its fruit has a high nutritional

value; especially ascorbic acid, dietary fibers and some antioxidant compounds (Jimenez-Escrig *et al.*, 2001).

In Thailand, major guava production areas of nearly 8,000 ha are located in the Central and Western parts of the country, especially Nakhon Pathom, Samut Sakhon, and Ratchaburi provinces; however, a guava plant can grow and produce fruits well in most regions in Thailand throughout the year. Prominent commercial cultivars are 'Paen Seethong', 'Klom Salee', and 'Yen Song'. These white flesh cultivars account for more than 90% of fresh guava consumption.

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At present, new cultivars with high nutritional value, excellent flavor, tolerant to biotic and abiotic stresses are increasingly important.

Major fruit qualities are quantitative traits and the phenotypic expression is complex. Knowledge of genetic and environmental factors that influence their phenotypic expressions is fundamental for a successful breeding program. The phenotypic variance can be partitioned into variances within and between individuals when a trait is repeatedly measured on each individual. Repeatability is a ratio of the between individual variance to the phenotypic variance. Repeatability estimates are useful for making predictions on progress in measurement, determining an upper limit of heritability, and predicting future performance from past records (Becker, 1984; Falconer and Mackay, 1996). Knowledge of the repeatability of quantitative traits helps in selecting efficient breeding strategies, including optimal sample size and evaluation methods. Several fruit breeding programs such as persimmon (Yamada *et al.*, 1993), strawberry (Sacks and Shaw, 1994), apricot (Akca and Sen, 1995), and peach (De Souza *et al.*, 1998) used the benefits of repeatability.

In the present research, the repeatability, optimal sample size, and phenotypic correlations of guava fruit traits were estimated to provide quantitative genetic information for guava breeding programs.

## MATERIALS AND METHODS

### Experimental materials

Eleven randomly selected guava clones consisted of six white flesh dessert types ('Klorn Salee', 'Khoa Um-porn', 'Yen Song', 'Paen Yak', 'Paen Seethong' and 'Na Suan'), one pink flesh dessert type ('Keynok Daeng'), two maroon flesh dessert types ('Daeng Siam' and 'Philippines'), and two pink flesh processing types ('MCL-326-S' and 'PC 12-102') from the guava germplasm

collection of the Department of Horticulture, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand were used. Guava trees were randomly planted in an experimental field (14°01'N lat., 99°58'E lon.) in December 1999, at a 4.0 m × 4.0 m spacing. The environmental conditions in the dry season (November to February) and the early rainy season (March to June) in 2001 had daily average max/min air temperature of 31.8/20.8°C and 33.6/24.5°C, daily average max/min RH of 95/50% and 95/58%, total precipitation of 9 mm and 265 mm, and daily average saturated light duration of 7.5 h d<sup>-1</sup> and 6.5 h d<sup>-1</sup>, respectively.

### Sampling methods

Fruit thinning by leaving one fruit per shoot was done in order to minimize the effects of over-cropping on fruit qualities such as size and sugar contents. Five fruits were randomly sampled from the same tree of each genotype in dry and early rainy season when the trees were 14 and 18 months old, respectively. In general, guava trees propagated by air-layering or cutting begin to set fruits in two to three months after planting but most growers do not allow trees to set fruits until six to eight months old. The changing in skin color was used as harvesting indicator. White flesh fruits were harvested when their skin color changed from dark green to light green, maroon flesh fruits were harvested when their skin color changed from dark maroon to light maroon, and processing types were harvested when their skin color changed from dark green to yellow green.

### Fruit quality measurements

Five physical fruit traits: fruit weight (FW), flesh thickness (FLT), flesh weight (FLW), seed cavity (central pulp) weight (SCW), fruit firmness (FF), and four chemical fruit traits: total soluble solids (TSS), titratable acidity (TA), juice acidity (pH), and ascorbic acid (AA) were evaluated. FW (g) and SCW (g) were measured

by digital balance (SK-5001, A&D, Japan). FLT (cm) was measured at equatorial plane with a caliper. FLW (g) was calculated by subtracting FW with SCW. FF (Newton; N) was determined on one side of fruit with fruit hardness tester (N.O.W., Japan) using 0.5 cm diameter probe after 0.3 cm skin was sliced off. Extracted juice from a flesh portion was used for determining the chemical traits. TSS was measured as °Brix with a temperature compensated hand refractometer (ATC-1E, Atago, Japan). TA (%) was determined by titration with 1.0 N NaOH and 1% phenolphthalein as an indicator using a digital burette (Burette digital III, Brand, Germany). The pH was determined using pH meter (pHScan 2, Eutech, Singapore). AA (mg) was estimated with oxalo-acetic acid solution and titration with 2, 6-dichlorophenolindophenol-dye solution (A.O.A.C., 1990).

### Statistical analysis

Data from each season was analyzed as a completely randomized design. An appropriate statistical model for expressing the phenotypic value of a trait is  $P_{ij} = \mu + g_i + f_{ij}$  (Becker, 1984). Where  $P_{ij}$  is the phenotypic value of the  $j^{\text{th}}$  fruit of the  $i^{\text{th}}$  genotype,  $\mu$  is the overall mean,  $g_i$  is the random effect of the  $i^{\text{th}}$  genotype, and  $f_{ij}$  is the random effect of  $j^{\text{th}}$  fruit in the  $i^{\text{th}}$  genotype. The repeatability of the guava fruit traits was estimated using one-way analysis of variance procedure (Becker, 1984). The formula is written as

$$\text{Repeatability} = \frac{\sigma_B^2}{\sigma_B^2 + \sigma_E^2}$$

where

$\sigma_B^2$  is the between genotypic variance and  $\sigma_E^2$  is the within genotypic variance.

with standard error of repeatability

$$\text{S.E.} = \sqrt{\frac{[2(1-R)^2][1+(k-1)R]^2}{k(k-1)(n-1)}}$$

Where  $k$  is the number of measurements (fruits)

per genotype,  $n$  is the number of genotypes, and  $R$  is the repeatability value.

The relative efficiency of measurements was estimated to obtain the optimal sample size for evaluating guava fruit traits. The formula is

$$\text{Relative efficiency} = \frac{k}{1+(k-1)R}$$

Where  $k$  is the number of measurements (fruits) and  $R$  is the repeatability value.

In this research, optimal sample size was selected when the relative efficiency increased by less than 10% with an additional measurement. The phenotypic correlations among traits were estimated on a cultivar mean basis from two seasons using Pearson's correlation coefficient ( $r$ ) analysis.

## RESULTS AND DISCUSSION

### Variance components

The phenotypic variance ( $\sigma_p^2$ ) of guava fruit traits in the dry and the early rainy seasons was different (Table 1), indicating that seasonal environmental conditions influenced the phenotypic expression of guava fruit qualities. The combined analysis of variance (ANOVA) over seasons confirmed that several traits, especially the chemical traits, were affected by seasons (Table 2). Therefore, it could be concluded that genetic expressions of chemical traits were highly sensitive to the changing of seasonal environments probably temperature and precipitation because these were clearly different between the two seasons as previously described in materials and methods. Rathore (1976) has reported that guava fruits harvested in spring, rainy and winter seasons in India had different levels of several chemical traits with rainy season fruits showing the lowest levels due to the fruits having the highest moisture contents. Effects of temperature on chemical compounds were also reported in several fruit crops such as apple (Hauagge and Cummins,

2000), grapevine (Lavee, 2000), peach (George and Erez, 2000).

Cultivars were different in most traits (Table 3) reflecting their difference in genetic background. The between genotypic variance ( $\sigma^2_B$ ) in most traits was higher than the within genotypic variance ( $\sigma^2_E$ ) in both seasons. The  $\sigma^2_B$  consists of genotypic variance ( $V_G$ ) + general environmental variance ( $V_{Eg}$ ) whereas the  $\sigma^2_E$  is the specific environmental variance ( $V_{Es}$ ) or sampling error associating with fruit set on different dates of the same plant. In this case,  $V_{Eg}$  referred to the seasonal environmental conditions such as temperature, precipitation, relative humidity, and light duration, while  $V_{Es}$  referred to the position and maturity stage of each fruit on the plant. Thus, guava fruit traits were influenced more by the seasonal environmental conditions than the fruit position or fruit maturity. In addition,  $\sigma^2_B$  was higher than  $\sigma^2_E$  in part due to the diverse guava cultivars used in this experiment (Table 4). Based on the ANOVA from Table 3, major part of  $\sigma^2_B$  of FW, FLT, FLW, and SCW could be the effect of  $V_G$ , whereas of FF, TSS, pH, and AA could be the effects of  $V_{Eg}$ .

## Repeatability

The repeatability of guava fruit traits in the dry and the early rainy seasons for most traits were relatively high (Table 1). Guava had higher repeatability than apricot for FW and TSS (Akca and Sen, 1995), peach for FW, TSS, and TA (De Souza *et al.*, 1998). This high repeatability in guava cultivars could be in part due to the diverse nature of their genetic background. To test this hypothesis, six commercial cultivars only from the white flesh dessert type ('Klom Salee', 'Khoa Um-porn', 'Yen Song', 'Paen Yak', 'Paen Seethong', and 'Na Suan') were used for repeatability estimation. This analysis yielded lower repeatability estimates than the 11 cultivars analysis (data not presented). The lower repeatability estimates for fruit traits from the commercial white flesh dessert type indicated that the genetic variance among these cultivars was small and consequently, guava breeding programs should include guava cultivars from other types such as processing cultivars and native cultivars to increase genetic variation of the breeding materials and to increase genetic gain in breeding program. Repeatability also establishes the upper limits of heritability (Becker 1984; Falconer and Mackay, 1996). Therefore, the

**Table 1** Variance components, repeatability (R), and standard error (S.E.) of repeatability of guava fruit traits in dry and early rainy seasons.

Trait <sup>1</sup>	$\sigma^2_P$		$\sigma^2_B$		$\sigma^2_E$		R $\pm$ S.E.	
	Dry	Early rainy	Dry	Early rainy	Dry	Early rainy	Dry	Early rainy
FW	68,027	65,260	47,214	52,684	20,813	12,575	0.69 $\pm$ 0.11	0.81 $\pm$ 0.08
FLT	0.39	0.59	0.33	0.50	0.06	0.09	0.85 $\pm$ 0.07	0.85 $\pm$ 0.07
FLW	48,247	45,996	33,305	37,152	14,942	8,844	0.69 $\pm$ 0.12	0.81 $\pm$ 0.08
SCW	1,961	2,617	1,282	1,944	678	673	0.65 $\pm$ 0.12	0.74 $\pm$ 0.10
FF	53.5	81.7	2.4	33.1	51.2	48.6	0.04 $\pm$ 0.11	0.40 $\pm$ 0.15
TSS	2.34	1.66	1.22	0.82	1.12	0.84	0.52 $\pm$ 0.15	0.49 $\pm$ 0.15
TA	0.33	0.12	0.28	0.10	0.05	0.02	0.85 $\pm$ 0.07	0.83 $\pm$ 0.07
pH	0.15	0.19	0.13	0.16	0.02	0.03	0.87 $\pm$ 0.06	0.84 $\pm$ 0.07
AA	2,745	1,420	2,099	1,141	645	280	0.76 $\pm$ 0.09	0.80 $\pm$ 0.08

<sup>1</sup> FW = fruit weight, FLT = flesh thickness, FLW = flesh weight, SCW = seed cavity weight, FF = fruit firmness, TSS = total soluble solids, TA = titratable acidity, pH = juice acidity, and AA = ascorbic acid.

**Table 2** Analysis of variance showing mean squares, and probabilities of test statistics for guava fruit traits.

Designation	df	Mean square	Probability
Fruit weight			
Cultivar	10	495,380	<0.01
Season	1	121,778	0.10
Cultivar x season	10	374,971	0.02
Residual	88	16,694	
Flesh weight			
Cultivar	10	345,143	<0.01
Season	1	59,114	0.20
Cultivar x season	10	30,928	0.01
Residual	88	11,893	
Flesh thickness			
Cultivar	10	3.72	<0.01
Season	1	0.20	0.55
Cultivar x season	10	0.51	<0.01
Residual	88	0.08	
Seed cavity weight			
Cultivar	10	15,989	<0.01
Season	1	11,201	0.02
Cultivar x season	10	1,496	0.02
Residual	88	676	
Fruit firmness			
Cultivar	10	188	0.13
Season	1	2,216	<0.01
Cultivar x season	10	89.2	0.07
Residual	88	49.9	
Total soluble solids			
Cultivar	10	7.3	0.27
Season	1	37.9	0.02
Cultivar x season	10	4.9	<0.01
Residual	88	1.0	
Titrateable acidity			
Cultivar	10	1.84	<0.01
Season	1	1.42	0.01
Cultivar x season	10	0.15	<0.01
Residual	88	0.03	
Juice acidity			
Cultivar	10	1.65	<0.01
Season	1	3.90	<0.01
Cultivar x season	10	0.04	0.09
Residual	88	0.02	
Ascorbic acid			
Cultivar	10	13,822	0.02
Season	1	15,868	0.05
Cultivar x season	10	3,303	<0.01
Residual	88	462	

repeatability of FW, FLT, FLW, SCW, TA, pH, and AA was relatively high for both seasons; actual heritability estimates for these traits would be expected to be relatively high. Similarly, the repeatability of FF and TSS was small for both seasons indicating that heritability estimates for these traits would also be relatively low. Based on the estimates of heritability, improving FF and TSS through selective breeding would be harder than for FW, FLT, FLW, SCW, TA, pH, and AA.

### Optimal sample size

The repeatability of a trait is used to estimate the relative efficiency of measurement to determine the optimal sample size (Becker, 1984). The relative efficiency of trait measurements with high repeatability was low, while those with low repeatability were high (Table 4). According to the formula, the relative efficiency with one fruit was 100%. The optimal sample size of measurement was determined when the increase in relative efficiency of measurement

**Table 3** Mean and standard error of fruit traits<sup>1</sup> in 11 guavas.

Name	Type	FW (g)	FLT (cm)	FLW (g)	SCW (g)	FF (N)
Klom Salee	Dessert	585 ± 79	2.8 ± 0.1	502 ± 67	83.1 ± 14.3	26.8 ± 2.9
Khoa Um-porn	Dessert	674 ± 30	3.1 ± 0.2	584 ± 28	89.6 ± 9.5	33.5 ± 2.6
Yen Song	Dessert	746 ± 39	2.6 ± 0.1	592 ± 31	153.4 ± 10.7	33.0 ± 1.8
Paen Yak	Dessert	640 ± 75	2.4 ± 0.1	523 ± 62	117.5 ± 14.5	32.8 ± 2.1
Paen Seethong	Dessert	716 ± 69	2.6 ± 0.1	582 ± 61	131.8 ± 12.8	31.6 ± 1.6
Na Suan	Dessert	526 ± 27	2.6 ± 0.1	445 ± 25	81.6 ± 5.3	33.7 ± 2.2
Keynok Daeng	Dessert	142 ± 10	1.3 ± 0.0	113 ± 8	29.2 ± 2.3	28.6 ± 4.2
Daeng Siam	Dessert	388 ± 29	2.1 ± 0.1	323 ± 25	64.9 ± 6.3	27.6 ± 2.2
Philippines	Dessert	304 ± 26	1.7 ± 0.0	239 ± 20	65.7 ± 7.1	24.9 ± 1.9
MCL-326-S	Processing	381 ± 18	2.0 ± 0.1	316 ± 16	65.0 ± 6.3	20.5 ± 2.8
PC 12-102	Processing	118 ± 8	1.2 ± 0.0	94 ± 6	23.2 ± 2.4	25.4 ± 4.5

<sup>1</sup> FW = fruit weight, FLT = flesh thickness, FLW = flesh weight, SCW = seed cavity weight, FF = fruit firmness.

**Table 3** Mean and standard error of fruit traits<sup>1</sup> in 11 guavas (continued).

Name	Type	TSS (°Brix)	TA (%)	pH	AA (mg)
Klom Salee	Dessert	6.8 ± 0.2	0.31 ± 0.05	4.3 ± 0.1	133 ± 9
Khoa Um-porn	Dessert	6.5 ± 0.4	0.38 ± 0.07	4.3 ± 0.1	120 ± 11
Yen Song	Dessert	7.4 ± 0.8	0.39 ± 0.07	4.3 ± 0.1	115 ± 12
Paen Yak	Dessert	7.1 ± 0.4	0.31 ± 0.03	4.3 ± 0.1	70 ± 8
Paen Seethong	Dessert	7.2 ± 0.5	0.39 ± 0.04	4.2 ± 0.1	68 ± 8
Na Suan	Dessert	7.0 ± 0.3	0.30 ± 0.02	4.4 ± 0.1	87 ± 7
Keynok Daeng	Dessert	9.4 ± 0.4	0.84 ± 0.07	3.8 ± 0.0	56 ± 2
Daeng Siam	Dessert	6.8 ± 0.3	0.40 ± 0.03	4.2 ± 0.1	126 ± 6
Philippines	Dessert	7.5 ± 0.4	0.37 ± 0.05	4.5 ± 0.1	82 ± 7
MCL-326-S	Processing	7.0 ± 0.4	1.75 ± 0.20	3.1 ± 0.1	119 ± 9
PC 12-102	Processing	8.6 ± 0.5	0.56 ± 0.05	4.0 ± 0.1	184 ± 17

<sup>1</sup> TSS = total soluble solids, TA = titratable acidity, pH = juice acidity, and AA = ascorbic acid.

was less than 10% when an additional measurement was done. The optimal sample size for most traits in both seasons was about three fruits except for FF. The FF needed 51 and six fruits per genotype in the dry and the early rainy seasons, respectively to reach the same accuracy.

### Phenotypic correlations

Phenotypic correlations as determined by the Pearson's correlation coefficient ( $r$ ) analysis between two traits may result from genetic associations due to linkage or pleiotropy (Falconer and Mackay, 1996). The four physical fruit traits;

FW, FLT, FLW, and SCW were strongly positively correlated ( $r \geq 0.80$ ) among themselves except for FLT with SCW which was moderately positively correlated ( $r = 0.50$ ) (Table 5). Therefore, using FW as a guide to screen for FLT, FLW, and SCW was a possibility since selection for higher FW should result in an increase in FLT, FLW, and SCW. However, selection for larger fruit may increase SCW and FW equally because the correlations of FW with SCW ( $r = 0.88$ ) and with FLT ( $r = 0.81$ ) were very similar. The correlations between all fruit traits associated with fruit size (FW, FLT, FLW, and SCW) with TSS and TA were negative

**Table 4** Relative efficiency of measurements and optimal sample size of guava fruit traits in dry and early rainy seasons.

Trait <sup>1</sup>	Relative efficiency with two fruits		Relative efficiency with three fruits		Optimal sample size	
	Dry	Early rainy	Dry	Early rainy	Dry	Early rainy
FW	1.18	1.11	1.26	1.14	3	3
FLT	1.08	1.08	1.11	1.11	2	2
FLW	1.18	1.11	1.26	1.14	3	3
SCW	1.21	1.15	1.30	1.21	3	3
FF	1.92	1.42	2.78	1.67	51	6
TSS	1.32	1.34	1.47	1.52	5	5
TA	1.08	1.09	1.11	1.13	2	2
pH	1.07	1.09	1.09	1.12	2	2
AA	1.13	1.11	1.19	1.15	3	3

<sup>1</sup> FW = fruit weight, FLT = flesh thickness, FLW = flesh weight, SCW = seed cavity weight, FF = fruit firmness, TSS = total soluble solids, TA = titratable acidity, pH = juice acidity, and AA = ascorbic acid.

**Table 5** Phenotypic correlations among guava fruit traits based on 11 cultivars in two seasons.

Trait <sup>1</sup>	FW	FLT	FLW	SCW	FF	TSS	TA	pH
FLT	0.81** <sup>2</sup>							
FLW	0.99**	0.85**						
SCW	0.88**	0.50**	0.83**					
FF	0.18 <sup>ns</sup>	0.11 <sup>ns</sup>	0.18 <sup>ns</sup>	0.17 <sup>ns</sup>				
TSS	-0.32**	-0.38**	-0.32**	-0.28**	0.08 <sup>ns</sup>			
TA	-0.27**	-0.26**	-0.27**	-0.27**	-0.05 <sup>ns</sup>	0.27**		
pH	0.32**	0.34**	0.32**	0.32**	0.00 <sup>ns</sup>	-0.36**	-0.84**	
AA	-0.14 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.11 <sup>ns</sup>	-0.25**	0.01 <sup>ns</sup>	0.22*	0.20*	-0.20*

<sup>1</sup> FW = fruit weight, FLT = flesh thickness, FLW = flesh weight, SCW = seed cavity weight, FF = fruit firmness, TSS = total soluble solids, TA = titratable acidity, pH = juice acidity, and AA = ascorbic acid.

<sup>2</sup> ns, \*, \*\* are non significant and significant at  $p \leq 0.05$  and  $0.01$ , respectively.



(Table 5) indicating that selection for fruit size might reduce TSS or TA. Therefore, improving of fruit size and TAA or TA may be carried out in separate crossing plan and combining these traits later. However, the correlations between all fruit traits associated with fruit size with TSS and TA were quite low ( $-0.26 \leq r \leq -0.38$ ), thus probably not of much practical importance. Three chemical fruit traits; TSS, TA, and AA were weakly positively correlated ( $0.20 \leq r \leq 0.27$ ) among themselves, while these three traits were negatively correlated with pH. Most of physical traits, especially FW had no correlation with AA. One objective of this guava-breeding program is to develop new cultivars with larger fruit and high ascorbic acid. These results indicated that selection for large fruit with high ascorbic acid was feasible.

### CONCLUSION

Repeatability estimates for FW, FLT, FLW, SCW, TA, pH, and AA were relatively high, indicating that response to selection for these traits would be realized in breeding program. Generally, three fruits per genotype provided sufficient efficiency for evaluating guava fruit traits. Most chemical traits had weak positive or negative correlation with fruit size, suggesting that early screening for chemical traits could be assayed indirectly using FW.

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