Optimal Spatial and Temporal Measurement Repetition for Selection in Japanese Persimmon Breeding

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Abstract Genetic and environmental variances for fruit ripening time (FRT), fruit weight, and soluble solids concentration (SSC) in Japanese persimmon (*Diospyros kaki* Thunb.) were estimated. The variance among fruit within a tree was the largest among environmental variances. Therefore, using a large sample size per tree and per year effectively increased heritability, but the effect was minimal when more than five fruit were sampled. The variance among years was largest for SSC and smallest for FRT. Generally, the variance associated with the genotype $\times$ year interaction was as large as the tree $\times$ year interaction. The variance among trees within a genotype was negligible for all traits. Repeating measurements yearly was more efficient than replicating trees.

In plant breeding, many seedlings must be tested to increase the chance of getting desirable genotypes. However, the number of seedlings used in fruit tree breeding is more restricted than in annual crop breeding, since fruit trees are large and occupy a large area. Success rate and cost are almost directly related to the space the progenies occupy and the amount of time they occupy that space (Hansche, 1983). Evaluating and selecting seedlings early decreases generation time and increases the scale of breeding work.

Most commercially important fruit traits, such as fruit ripening time (FRT) and fruit weight (FW), are quantitative, and their performance fluctuates due to environmental factors. Therefore, it is important to obtain information on the contribution of genetic and environmental factors to phenotypic expression of such traits (Hansche and Beres, 1966; Hansche and Brooks, 1965; Machida and Kozaki, 1975; Yamada and Kurihara, 1984; Yamada et al., 1986).

Increasing the number of tree replications, years, and fruit samples would increase measurement accuracy. Estimated genetic and environmental variances for fruit traits could provide the optimal sample size, yearly repetition, or replication number to effectively dis-

criminate genetic differences for traits.

Reports on crops other than fruit (Kaltsikes, 1970; Mycroft, 1983; Patterson et al., 1977; Rasmussen and Lambert, 1961; Sekioka and Lauer, 1970; Shorter and Norman, 1983; Swallow and Wehner, 1989) indicated that adding more locations, years, or both was more efficient than adding replications.

The aim of this study was to estimate genetic and environmental variances for three fruit traits in Japanese persimmon and to provide the optimal sample size, yearly repetition, or number of tree replications to obtain the highest efficiency for evaluating genetic differences.

These investigations were conducted at the Fruit Tree Research Station, Akitsu, Japan, using genotypes of pollination-constant (Hume, 1914) and nonastringent Japanese persimmons. The trees used for the experiment were 15 years old in 1982.

Variance estimation with 2 years, eight genotypes, and three trees. In a study with three trees each of eight genotypes, one branch with 25 to 148 fruit was sampled per tree. Fruit

were harvested at their respective FRT, which was when the fruit were ready to eat. FRT was judged by flesh firmness rated on a scale of 1 to 8. A rating of 1 = late September, 2 = early October, 3 = mid-October, 4 = late October, 5 = early November, 6 = mid-November, 7 = late November, and 8 = early December. FW and soluble solids concentration (SSC) also were evaluated. SSC was determined with a calibrated refractometer (model N1; Atago, Tokyo) on a small volume of fruit juice pressed out from the equatorial portion of fruit.

The measurements from the same number (25) of fruit taken randomly from each branch were subjected to analysis of variance as noted below. Before analysis, FW was log-transformed to improve the normality of the distribution of the observations. The genetic model adopted here for expressing the phenotypic value of a trait is $P_{ijkl} = \mu + g_i + t_j + f_{ijk} + y_l + (gy)_{il} + (ty)_{jl}$, where P_{ijkl} is the phenotypic value of the k th fruit of the j th tree of the i th genotype in the l th year, μ is the overall mean, g_i is a random effect contributed by the i th genotype, t_j is a random effect of the j th tree of the i th genotype, y_l is a random effect of the l th year, f_{ijk} is a random effect of the k th fruit of the j th tree of the i th genotype in the l th year, $(gy)_{il}$ is the interaction between the i th genotype and the l th year, and $(ty)_{jl}$ is the interaction between the j th tree of the i th genotype and the l th year.

Total variance (σ^2_T) in the analysis of variance was divided into the variance associated with genotype (σ^2_g), trees within genotype (σ^2_t), fruit samples within a tree (σ^2_f), year (σ^2_y), the genotype $\times$ year interaction (σ^2_{gy}), and the tree $\times$ year interaction (σ^2_{ty}).

As the result of analysis of variance, the effects of genotype, year, the genotype $\times$ year interaction, and the tree $\times$ year interaction were significant at $P \leq 0.01$ for all three fruit traits, with the exception of year and genotype $\times$ year interaction for FRT, for which the year effect was very small (Table 1). The effect of tree within genotype was nonsignificant for all traits. The ratio of the genetic variance to the total variance was highest for FRT and lowest for SSC (Table 2).

The contribution of spatial environmental variance components (σ^2_t , σ^2_{ty} , σ^2_f) to σ^2_T was similar among fruit traits. Among trees, σ^2_t was negligibly small. The σ^2_f was largest among

Table 1. Analysis of variance for fruit traits of Japanese persimmon using eight genotypes with three trees per genotype for 2 years.

Source of variation	df	Fruit trait (mean square)			Expected mean squares
		Fruit ripening time	Fruit wt ² ($\times 10^{-3}$)	Soluble solids concn	
Genotype	7	501.48**	12.52**	155.92**	$\sigma^2_g + 25 \sigma^2_{ty} + 75 \sigma^2_{gy} + 50 \sigma^2_t + 150 \sigma^2_f$
Year	1	16.05 ^{ns}	10.52**	761.02**	$\sigma^2_y + 25 \sigma^2_{ty} + 75 \sigma^2_{gy} + 600 \sigma^2_f$
Genotype $\times$ year	7	9.43 ^{ns}	1.20**	80.65 ^{ns}	$\sigma^2_g + 25 \sigma^2_{ty} + 75 \sigma^2_{gy}$
Among trees					
within genotype	16	3.59 ^{ns}	0.23 ^{ns}	8.92 ^{ns}	$\sigma^2_t + 25 \sigma^2_{ty} + 50 \sigma^2_f$
Tree $\times$ year	16	4.18**	0.16**	5.96**	$\sigma^2_t + 25 \sigma^2_{ty}$
Among fruit					
within tree	1152	0.49	0.04	1.70	σ^2_f

²Fruit weight was logarithmically transformed.

^{ns},**Nonsignificant or significant at $P \leq 0.01$, respectively, using F test.

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Table 2. Estimates of variance components and their percentage to total variance obtained from the analysis of variance for Japanese persimmon fruit traits using eight genotypes with three trees per genotype for 2 years.

Variance components	Fruit trait (mean square)		
	Fruit ripening time	Fruit wt ² (×10 ⁻³)	Soluble solids concn
σ^2	3.28 (82.0%)	7.50 (49.4%)	0.48 (10.6%)
σ^2_{g}	0.01 (0.3%)	1.55 (10.2%)	1.13 (24.9%)
σ^2_{y}	0.07 (1.8%)	1.39 (9.2%)	0.99 (21.9%)
$\sigma^2_{\text{g} \times \text{y}}$	0 ^a (0%)	0.13 (0.9%)	0.06 (1.3%)
σ^2_{e}	0.15 (3.7%)	0.50 (3.3%)	0.17 (3.8%)
σ^2_{f}	0.49 (12.2%)	4.10 (27.0%)	1.70 (37.5%)
σ^2_{t}	4.00 (100.0%)	15.17 (100.0%)	4.53 (100.0%)

^aFruit weight was logarithmically transformed.

^bNegative-values were assumed to be zero.

the three components, ranging from 12.2% to 37.5%. The σ^2_{y} was ≈3% to 4% of the total variance. The σ^2_{g} was as large as σ^2_{f} , large compared with σ^2_{y} for FW and SSC, but very small (smaller than σ^2_{y}) for FRT.

Variance estimation with 9 years, 19 genotypes, and one tree. Nineteen genotypes, including the eight from the previous experiment, with a single tree per genotype were used in this experiment (Table 3). Fifteen fruit were taken from each tree and examined for the three fruit traits for 9 years from 1982 to 1990.

The mean values of each genotype and year were subjected to analysis of variance using the following model: $P'_{ij} = \mu' + g'_i + y'_j + e'_{ij}$, where P'_{ij} is the phenotypic value of the i th genotype in the j th year, μ' is the overall mean, g'_i is a random effect of the i th genotype, y'_j is a random effect of the j th year, and e'_{ij} is the residual effect of i th genotype and j th year. We assumed that all random effects had means of zero and were independent of each other.

The total variance (σ^2_{t}) was divided into σ^2_{g} , σ^2_{y} , and residual (σ^2_{e}). According to the analysis of variance, the effects of genotype and year were significant ($P \leq 0.01$) for all

Table 3. The 19 Japanese persimmon genotypes evaluated over 9 years.

Genotype	Origin
F-2	F ₁ of Jirou x Okugosho
Fuyuu ^a	Native cultivar in Japan
Hanagosho	Native cultivar in Japan
Ichikikeijirou	Bud mutant of Jirou
Izu ^a	F ₁ of Fuyuu x Okitsu-1
Izushiohgoshoh	Native cultivar in Japan
Jirou ^a	Native cultivar in Japan
Maekawajirou ^a	Bud mutant of Jirou
Matsumotowasefuyuu	Bud mutant of Fuyuu
Okitsu-1 ^a	F ₁ of Okugosho selfing
Okitsu-14	F ₁ of Okugosho x Hanagosho
Okitsu-16	F ₁ of Okugosho x Hanagosho
Okitsu-20	F ₁ of Fukurogoshoh x Hanagosho
Okitsu-21	F ₁ of Jirou x Tenjingoshoh
Okitsu-24	F ₁ of Jirou x Okitsu-15 ^a
Okitsu-25	F ₁ of Fuyuu x F-2
Okugosho ^a	Native cultivar in Japan
Suruga ^a	F ₁ of Hanagosho x Okugosho
Tenjingoshoh	Native cultivar in Japan

The eight genotypes used in the previous analysis (Table 2) in which they were measured with three-tree replicates for 2 years.

^aF₁ of Okugosho x Hanagosho.

three fruit traits (Table 4). Year-to-year variability was divided into σ^2_{y} and σ^2_{e} . Estimates of variance components are listed in Table 5. The ratio of σ^2_{g} to the total variance was highest for FRT and lowest for SSC. The σ^2_{y} was largest for FW and lowest for FRT.

The σ^2_{g} was composed of among-tree variance and the true genetic variance (σ^2_{g}), which was obtained by subtracting σ^2_{t} (estimated in the previous analysis of variance using eight genotypes) from σ^2_{g} .

The σ^2_{e} consisted of σ^2_{y} , σ^2_{f} /n (where n is the number of fruit examined per year), and the true variance for the genotype × year interaction ($\sigma^2_{\text{g} \times \text{y}}$). The $\sigma^2_{\text{g} \times \text{y}}$ was obtained by subtracting σ^2_{y} and σ^2_{f} /n (estimated in the previous analysis) from σ^2_{e} .

Higher precision can be expected for $\sigma^2_{\text{g} \times \text{y}}$ than σ^2_{y} because there are more degrees of freedom for the former. The diverse environmental variance components were estimated using $\sigma^2_{\text{g} \times \text{y}}$ as the genotype × year interaction variance (Table 6), which was as large as σ^2_{y} .

Heritability. Heritability (h^2) would increase if the environmental variance components decreased, for example, by removing the year effect. The year effect can be estimated by the yearly deviation in mean performance of many control genotypes that are measured every year (M. Yamada and Y. Ukai, unpublished data). In such a case, the environmental variance of the mean for the progenies concerned may be expressed as $\sigma^2_{\text{e}} = \sigma^2_{\text{g} \times \text{y}}/y + \sigma^2_{\text{f}}/(yt) + (\sigma^2_{\text{t}}/t) + \sigma^2_{\text{f}}/(ytf)$, where σ^2_{e} is the total environmental variance of mean performance in the progeny concerned, y is the number of years repeated, t is the number of trees, and f is the number of fruit per tree and year.

The h^2 estimates were obtained by the following relation: $h^2 = \sigma^2_{\text{g}}/(\sigma^2_{\text{g}} + \sigma^2_{\text{e}})$, where σ^2_{g} is the environmental variance and σ^2_{e} is the

Table 5. Estimates of variance components obtained from the analysis of variance for Japanese persimmon fruit traits using 19 genotypes with a single tree per genotype for 9 years.

Variance components	Fruit trait (mean square)		
	Fruit ripening time	Fruit wt ² (×10 ⁻³)	Soluble solids concn
Genotype (σ^2_{g})	1.65	4.19	0.72
Year (σ^2_{y})	0.03	0.57	0.25
Residual (σ^2_{e})	0.25	1.23	0.47

^aFruit weight was logarithmically transformed.

Table 6. Estimates of variance components obtained from the analyses of variance using eight genotypes for 2 years and 19 genotypes for 9 years.

Variance components	Fruit trait (mean square)		
	Fruit ripening time	Fruit wt ² (×10 ⁻³)	Soluble solids concn
σ^2_{g}	1.65	4.06	0.66
$\sigma^2_{\text{g} \times \text{y}}$	0.07	0.45	0.19
σ^2_{e}	0	0.13	0.06
σ^2_{y}	0.15	0.50	0.17
σ^2_{f}	0.49	4.10	1.70

^aFruit weight was logarithmically transformed.

$\sigma^2_{\text{g} \times \text{y}} = \sigma^2_{\text{g}} - \sigma^2_{\text{y}}$.

$\sigma^2_{\text{e}} = \sigma^2_{\text{e}} - \sigma^2_{\text{y}} - \sigma^2_{\text{f}}/n$; n = 15, which is the number of fruit examined per tree and year in the analysis for 19 genotypes measured for 9 years.

genetic variance of the progenies. Here, σ^2_{g} was replaced by σ^2_{g} .

Optimum repetition. The optimum number of tree replications and yearly repetitions to efficiently estimate genetic variability in breeding stocks can be calculated using values of environmental variance components (Table 6).

In breeding Japanese persimmon at the Akitsu Fruit Tree Research Station, top-grafting techniques have been adopted to save space in the field and shorten the juvenile phase of trees. In this case, the number of replicates per seedling progenies to be top-grafted greatly influenced breeding efficiency. Here, t is the number of trees top-grafted. During the second or third fruiting year, trees usually produced ≤ 10 fruit when one progeny was top-grafted to a single branch of a mature tree. For trees to bear ≥ 20 fruit in early fruiting years, top-grafting one progeny to two or more branches is required. The σ^2_{e} may be smaller when top-grafting to different trees than top-grafting to the same tree, since: σ^2_{y}/yt , σ^2_{f}/t , and σ^2_{f}/ytf are expected to be smaller in the former. Therefore, the repetition of >10 fruit per tree was not considered here.

Nineteen genotypes used in this experi-

Table 4. Analysis of variance for fruit traits of Japanese persimmon using 19 genotypes with a single tree per genotype for 9 years.

Source of variation	df	Fruit trait (mean square)			Expected mean squares
		Fruit ripening time	Fruit wt ² (×10 ⁻³)	Soluble solids concn	
Genotype	18	15.07**	38.90**	6.92**	$\sigma^2_{\text{g}} + 9 \sigma^2_{\text{e}}$
Year	8	0.89**	12.07**	5.25**	$\sigma^2_{\text{e}} + 19 \sigma^2_{\text{y}}$
Residual	144	0.25	1.23	0.46	σ^2_{e}

^aFruit weight was logarithmically transformed.

**Significant at $P \leq 0.01$.

ment were parents used in Japanese persimmon breeding programs. The σ_g^2 was estimated from the σ_{ϵ}^2 of 19 genotypes measured for 9 years, where the mean was 5.62 for FRT, 234 g for FW, and 17.39% for SSC.

The influence of spatial and temporal measurement repetition on h^2 is shown in Fig. 1. This figure can be used to assess the efficacy of the number of replicates or yearly repetitions for discriminating genotypes.

For FRT, h^2 was 0.84, 0.90, and 0.92 for the cases where single-tree, two-tree, and three-tree replications, respectively, were used with five fruit without repeating years. Thus, h^2 was estimated high, even in the case of a single tree with five fruit, without repeating years. Multiple measurements increased accuracy little for this trait. The h^2 estimate was slightly higher for a single tree, five fruit, and 2 years than for two trees, five fruit, and a single year. The former was estimated as 0.91 and the latter as 0.90. To increase h^2 , an increase in the number of years instead of the number of tree replications was effective. We conclude that an increase in the number of fruit measured per tree and year increased accuracy little, because no clear difference in h^2 between 10 and five fruit was observed.

For FW, h^2 was 0.68 in a case of a single tree, five fruit, and a single year. The h^2 increased to 0.85 by repeating the measurement for 3 years. The increase in accuracy by spatial and temporal measurement repetition was greater for FW than for FRT. Yearly repetition gave a larger increase in h^2 than tree replication. The h^2 was 0.77 for two trees, five fruit, and a single year, and was higher than for a single tree, five fruit, and a single year by 0.09—slightly lower than for a single tree, five

fruit, and 2 years. The h^2 for three trees, five fruit, and a single year was 0.81—slightly lower than for a single tree, five fruit, and 3 years. Increasing the number of fruit from five to 10 slightly increased h^2 —0.68 to 0.72.

For SSC, h^2 was lower than for FW. The effect of yearly repetition on h^2 still was greater than FRT and FW. For a single tree and five fruit, h^2 was 0.47 for a single year and increased to 0.69 for 3 years. For SSC, yearly repetition also produced a greater increase in h^2 than tree replication, as was found in other traits. The increment obtained for SSC with yearly repetition was greatest among the three traits. For five fruit per tree and year, h^2 was higher for a single tree and 2 years than for two trees and a single year. Using 10 fruit and a single tree produced a higher h^2 than that for five fruit and a single tree but a lower h^2 than that of two trees and five fruit.

In summary, yearly repetition more efficiently increased h^2 than tree replication. Using 10 fruit per tree and year increased h^2 some, but not much compared with five fruit.

Cost considerations. Cost depends on the space in the field and the speed of selection. If r_1 is the number of interstocks to be top-grafted in a breeding field and r_2 is the number of branches in an interstock, then the total number of the branches to be top-grafted (r) can be shown as $r = r_1 \times r_2$. If Y is the number of years selection can be continued in a selection field in a breeding program, then the total number of progenies that can be tested can be expressed as $N = r \times Y$.

Suppose that the first fruiting year is the fourth year after top-grafting, as is normal with Japanese persimmon breeding at Akitsu. If y is the number of years for repeating

measurements and t is the number of trees to be top-grafted, then the total number of branches top-grafted over years that can be accommodated (T) is $T = N/[(3 + y) \times t]$. For the cycle in which top-grafting was done to a single tree and the selection was done in the first year of fruiting and examination, $T = N/(4 \times 1) = N/4$. Similarly, with top-grafting to a single tree and selection in the third fruiting year, $T = N/6$. However, when top-grafted to three branches on different interstocks and selection in the first fruiting year, $T = N/(4 \times 3) = N/12$.

Thus, an increase in the number of tree replications markedly reduced the total number of progenies that could be tested compared to an increase in years.

Conclusions. To compare the total number of progenies that can be field-tested, we have shown that using tree replications was less efficient than yearly repetitions. Unless σ_t^2 is markedly greater than σ_{gy}^2 , it may be disadvantageous to increase the number of tree replications while sacrificing yearly repetitions. However, σ_t^2 was smaller than σ_{gy}^2 for all fruit traits examined in the present study. An increase in yearly repetition instead of tree replications substantially reduced σ_{ϵ}^2 in the measurements and, hence, increased h^2 . This trend was pronounced for SSC and FW. In conclusion, field trials should be conducted over additional years rather than using more tree replications per year to maximize efficiency in Japanese persimmon breeding.

This conclusion is applicable mainly to first-stage selection on the total progeny population. Once a few genotypes have been selected, measurements should be obtained from many tree replications over many years to clarify the genetic properties of selected genotypes.

Fig. 1. Effects of tree replication, number of fruit, and yearly repetition for measurements on the heritability of fruit ripening time (FRT), fruit weight (FW,) and soluble solids concentration (SSC) of Japanese persimmon. Heritability was defined as $\sigma_g^2/(\sigma_g^2 + \sigma_{gy}^2/y + \sigma_{ty}^2/yt + \sigma_t^2/t + \sigma_{\epsilon}^2/yt)$ (see text for details). ● = one tree and five fruit, □ = one tree and 10 fruit, ○ = two trees and five fruit per tree, ▲ = three trees and five fruit per tree

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