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Optimal sampling strategies for evaluating fruit softening after harvest in apple breeding

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Summary

Environmental variance components associated with year, tree, and harvest date were estimated for fruit softening after harvest in apple (*Malus* × *domestica* Borkh.) to determine their relative importance and design optimum sampling strategies to discriminate genotypes in apple breeding. Fruit were stored after harvest under 20 ± 2 °C and 80 ± 5%RH. Softening was evaluated by adapting the change in firmness during storage to a linear regression and defining the regression coefficient as the softening rate. Environmental variances associated with genotype × year interaction, among trees, year × tree interaction, and among harvest dates were all very small, namely, 2.7, 0.1, 5.2, and 5.7%, respectively, to the total variance obtained from the analysis of variance for the softening rate. The variance associated with genotype, at 57.3%, was very large. On the basis of the number of fruit necessary for firmness measurements, two times harvest is an efficient strategy to determine a genotype mean for the softening.

Introduction

Softening of fruit after harvest is a serious problem for apple (*Malus* × *domestica* Borkh.) growers and flesh markets. Since apples are not always marketed directly after harvest (Kingston, 1992), growers and markets are quite concerned about the length of time that fruit retains its firmness, which is directly linked to consumer acceptance. Many studies, consequently, have attempted to predict changes in fruit firmness from harvest to after or during storage using regression equations by measuring quality at harvest (Ingle et al., 2000; Johnson & Ridout, 1998; Blankenship et al., 1997; Evensen et al., 1993; Ingle & Morris, 1989) or to determine harvest indicators that have a close relationship to firmness after storage (Ingle & D'Souza, 1989; Knee & Smith, 1989).

Apples do not always soften continuously during storage, and the extent of softening after harvest is greatly influenced by the storage period (Iwanami et al., 2004). Determining how long it takes for fruit to become too soft for market acceptance requires regular evaluation until firmness reaches a certain value, and, consequently, many samples of fruit are required when a genotype is likely to have a long storage capability. Therefore, although long shelf life is one of the most important objectives in apple breeding, this important part of the seedling screening is ignored in many apple-breeding programs (Alston, 1988). Recently, Iwanami et al. (2004) indicated that the firmness of apple fruit significantly decreased by 20 days of storage at 20 ± 2 °C and 80 ± 5%RH even in genotypes with a long storage potential. They proposed a method to evaluate genotype differences in softening using a regression coefficient of change in firmness during storage with restricted fruit samples. By comparing the regression coefficients of seedlings with those of control cultivars or among themselves, superior genotypes can be selected.

On the other hand, softening, estimated by the regression coefficient proposed by Iwanami et al. (2004), is a quantitative and phenotypic expression of the softening of a genotype, which can be compared with that of another genotype, can fluctuate depending on environmental factors such as year, location, and sampling date. Johnston et al. (2002) suggested that one of the major factors that influence post-harvest softening of apple fruit at harvest is their maturity. This means that a slight difference in the harvest date could greatly influence the phenotypic expression of the fruit softening of a genotype. Johnston et al. (2002) also suggested that fruit from different orchards often differ in firmness after storage despite being stored under similar conditions. Ingle and Morris (1989) observed that the rate of 'Rome' apple fruit softening at 20 °C varied from year to year. These previous reports, however, have not estimated the magnitudes of the environmental variances of fruit softening.

Environmental variances normally fluctuate depending on the location, climate, and cultural management. Therefore, it is important for apple breeders to obtain information on the magnitudes of the environmental variances under their cultural management and climate conditions and the contribution of genetic and environmental factors to phenotypic expression. The objectives of this study were (1) to estimate the environmental variance components of softening specific to apple breeding at the Department of Apple Research, National Institute of Fruit Tree Science (NIFTS) in Japan and (2) to determine the optimum sampling methods for reducing the environmental variation of fruit softening after harvest.

Materials and methods

Fruit materials

Thirteen apple cultivars and selections (genotypes) from the orchards at the Department of Apple Research of NIFTS were selected on the basis of diversity of harvest date (Table 1). The genotypes were used as parents in the NIFTS apple-breeding programs. Two or three trees from each genotype were used for the experiment. The trees varied in rootstock and years since planting/top-grafting. Each of 20 to 25 fruit samples was harvested three times weekly during the maturity period in 2001 and 2002. The maturity period was determined as the time when most of the fruits were considered to be mature based on ground color, texture, flavor and starch staining. Fruits of similar size (within 15% of average fruit weight) were harvested randomly from each tree. Four to five fruits from each harvested sample were immediately used for the measurement of firmness and soluble solids concentration (SSC). SSC was used to assess the degree of maturity of sampling fruit because SSC tends to increase as apple fruit mature and so it could be a useful maturity index for the fruit (Kingston, 1992). Flesh firmness was measured using a fruit pressure tester (FT327, McCormick Fruit Technology, Wash.) mounted in a drill press and fitted with an 11.1 mm probe on the pared surfaces of the sunny and shady sides of each fruit. Data were expressed in Newton (N). SSC was measured with a digital refractometer (PR-100, Atago, Tokyo) using crude juice extracted with a juicer (MJ-C68, National, Osaka) from each fruit that was peeled and cored.

Storage conditions

The remaining fruit was stored in 25-l containers arranged on racks in a chamber controlled at 20 ± 2 °C and 80 ± 5%RH with constant air circulation. The firmness of four to five fruit was measured at 5-day intervals until 20 days after harvest. Fruit displaying rot were immediately removed from the containers during storage.

Softening evaluation

Firmness measurements of individual fruit sampled were subjected to a linear regression on days of storage. Since firmness does not always decrease uniformly throughout 20 days of storage, the linear regression for change in firmness was adapted from the harvest date until firmness decreased by 20%, as described by Iwanami et al. (2004). When firmness did not decrease more than 20% within 20 days of storage, the data from the full length of storage (20 days) were subjected to linear regression. The regression coefficient, calculated by subjecting the firmness measurement to the linear regression, was defined as the softening rate (*N*/day). Softening was evaluated by the softening rate.

Statistical analysis

Comparison of the softening rate among harvest dates

The regression coefficients (softening rate) were compared according to Okuno (1978). Differences in the regression coefficients among harvest dates were detected by the following F-test:

$$F = \frac{\text{Mean squares among regressions}}{\text{Mean squares of regression residual}},$$

Table 1. Harvest date and storage periods of the fruit from 30 trees of 13 apple cultivars and selections in 2001 and 2002

Genotype	Rootstock	Tree age[a] in 2001	2001						2002					
			Harvest date			Storage periods[b] (day)			Harvest date			Storage periods[b] (day)		
			1	2	3	1	2	3	1	2	3	1	2	3
Sansa	M.26 EMLA	15	31 Aug.	7 Sept.	14 Sept.	10	10	10	30 Aug.	6 Sept.	13 Sept.	10	10	5
	M.9	7	31 Aug.	7 Sept.	14 Sept.	10	10	10	30 Aug.	6 Sept.	13 Sept.	10	10	5
	M.9	7	31 Aug.	7 Sept.	14 Sept.	10	10	10	30 Aug.	6 Sept.	13 Sept.	10	10	10
Tsugaru	JM7	7	31 Aug.	7 Sept.	14 Sept.	15	15	10	6 Sept.	13 Sept.	20 Sept.	10	10	10
	M.9	7	31 Aug.	7 Sept.	14 Sept.	15	15	10	6 Sept.	13 Sept.	20 Sept.	10	10	10
	M.9	7	31 Aug.	7 Sept.	14 Sept.	15	15	10	6 Sept.	13 Sept.	20 Sept.	10	10	10
Akane	M.26 EMLA	Unknown	31 Aug.	7 Sept.	14 Sept.	10	15	10	6 Sept.	13 Sept.	20 Sept.	5	5	5
	Jonathan/M.prunifolia	(3)	31 Aug.	7 Sept.	14 Sept.	10	10	10	6 Sept.	13 Sept.	20 Sept.	10	5	10
Silken	M.9	11	20 Sept.	28 Sept.	5 Oct.	10	10	10	13 Sept.	20 Sept.	27 Sept.	10	10	10
	M.9	11	20 Sept.	28 Sept.	5 Oct.	10	10	5	13 Sept.	20 Sept.	27 Sept.	10	10	10
Himekami	S.D./MM.106	(17)	14 Sept.	20 Sept.	28 Sept.	10	10	10	13 Sept.	20 Sept.	27 Sept.	10	10	10
	S.D./MM.106	(17)	14 Sept.	20 Sept.	28 Sept.	10	10	10	13 Sept.	20 Sept.	27 Sept.	10	10	10
Santaro	M.26	12	20 Sept.	28 Sept.	5 Oct.	10	15	10	20 Sept.	27 Sept.	5 Oct.	10	10	10
	M.26	12	20 Sept.	28 Sept.	5 Oct.	10	15	10	20 Sept.	27 Sept.	5 Oct.	10	10	10
Morioka57	JM2	11	28 Sept.	5 Oct.	11 Oct.	10	10	10	27 Sept.	5 Oct.	11 Oct.	5	5	5
	M.26	12	28 Sept.	5 Oct.	11 Oct.	10	10	10	27 Sept.	5 Oct.	11 Oct.	5	5	10
Jonathan	JM7	7	5 Oct.	11 Oct.	20 Oct.	10	10	10	5 Oct.	11 Oct.	17 Oct.	10	10	15
	M. prunifolia	43	5 Oct.	11 Oct.	20 Oct.	10	15	10	5 Oct.	11 Oct.	17 Oct.	10	10	10
Kotaro	JM2	11	20 Oct.	26 Oct.	2 Nov.	10	10	10	17 Oct.	25 Oct.	1 Nov.	10	10	10
	M.26	12	20 Oct.	26 Oct.	2 Nov.	15	15	10	17 Oct.	25 Oct.	1 Nov.	10	10	10
Golden	M.9	7	20 Oct.	26 Oct.	2 Nov.	10	10	10	17 Oct.	25 Oct.	1 Nov.	10	5	10
Delicious	M.9	7	20 Oct.	26 Oct.	2 Nov.	10	10	10	17 Oct.	25 Oct.	1 Nov.	10	5	10
Orin	JM7	7	20 Oct.	26 Oct.	2 Nov.	15	10	10	25 Oct.	1 Nov.	8 Nov.	10	10	10
	M.9	7	20 Oct.	26 Oct.	2 Nov.	15	10	10	25 Oct.	1 Nov.	8 Nov.	10	10	10
	M.9	7	20 Oct.	26 Oct.	2 Nov.	15	15	10	25 Oct.	1 Nov.	8 Nov.	10	10	10
Fuji	JM7	7	2 Nov.	9 Nov.	17 Nov.	20	20	20	1 Nov.	8 Nov.	16 Nov.	20	20	20
	M.9	7	2 Nov.	9 Nov.	17 Nov.	20	20	20	1 Nov.	8 Nov.	16 Nov.	20	20	20
	M.9	7	2 Nov.	9 Nov.	17 Nov.	20	20	20	1 Nov.	8 Nov.	16 Nov.	20	20	20
Ralls Janet	M.9	7	9 Nov.	17 Nov.	22 Nov.	10	15	15	8 Nov.	16 Nov.	23 Nov.	10	20	10
	M.9	7	9 Nov.	17 Nov.	22 Nov.	10	10	15	8 Nov.	16 Nov.	23 Nov.	10	15	10

S.D.: Starking Delicious.

[a] Numeral in parentheses indicates years since top-grafting on intermediate stock.

[b] Storage continued until the flesh firmness decreased by 20% of harvest firmness or for 20 days when the flesh firmness did not decrease more than 20% within 20 days of storage.

in which

$$\text{Mean squares among regressions} = \frac{\sum_{i=1}^{g}\sum_{j=1}^{t}\sum_{k=1}^{y}\left[\sum_{l=1}^{h}\left[(Sxy)^2_{ijkl}/(Sx)_{ijkl}\right] - \left\{\sum_{l=1}^{h}(Sxy)ijkl\right\}^2/\sum_{l=1}^{h}(Sx)_{ijkl}\right]}{\sum_{i=1}^{g}\sum_{j=1}^{t}\sum_{k=1}^{y}(l_{ijk}-1)}, \tag{1}$$

$$\text{Mean squares of regression residual} = \frac{\sum_{i=1}^{g}\sum_{j=1}^{t}\sum_{k=1}^{y}\sum_{l=1}^{h}(Se)_{ijkl}}{\sum_{i=1}^{g}\sum_{j=1}^{t}\sum_{k=1}^{y}\sum_{l=1}^{h}(m_{ijkl}-2)}, \tag{2}$$

where $(Sx)_{ijkl}$ is the sum of squares of independent variables (x: storage day) of the lth harvest date in the jth tree of the ith genotype in the kth year; $(Sxy)_{ijkl}$ the sum of products of variables x (storage day) and y (firmness) of the lth harvest date in the jth tree of the ith genotype in the kth year; $(Se)_{ijkl}$ the sum of squares of regression residual of the lth harvest date in the jth tree of the ith genotype in the kth year; l_{ijk} the number of harvest in the jth tree of the ith genotype in the kth year; m_{ijkl} the number of sampling fruit per harvest of the lth harvest date in the *j*th tree of the *i*th genotype in the *k*th year.

Comparison of the softening rates among genotypes, trees, and years

The regression coefficients from each harvest date were subjected to analysis of variance (ANOVA). The homogeneity of the variances among harvest dates within a tree was tested by Bartlett's test, and the normal distribution of the residual was tested using Kolmogorov–Smirnov's one-sample test. The results of these tests showed that the homogeneity of the variance and normal distribution was not rejected at $P = 0.05$, and, therefore, the model of ANOVA was assumed to be applicable to the data. The model adopted here to express the phenotypic value is $P_{ijkl} = \mu + g_i + t_{ij} + y_k + (gy)_{ik} + (ty)_{ijk} + h_{ijkl}$, where P_{ijkl} is the regression coefficient of the *l*th harvest date in the *j*th tree of the *i*th genotype in the *k*th year; μ the overall mean; g_i the random effect of the *i*th genotype; t_{ij} the random effect of the *j*th tree of the *i*th genotype; y_k the random effect of the *k*th year; $(gy)_{ik}$ the interaction between the *i*th genotype and the *k*th year; $(ty)_{ijk}$ the interaction between the *j*th tree of the *i*th genotype and the *k*th year; and h_{ijkl}, the random effect of the *l*th harvest date in the *j*th tree of the *i*th genotype in the *k*th year. ANOVA was performed using a Statistical Analysis System (SAS Institute, Cary, NC, 1989).

Estimation of the environmental variance components of the softening rate

The ANOVA provided the variance associated with the genotype (σ_{g}^2), among trees within genotypes (σ_{t}^2), among years (σ_{y}^2), the genotype × year (σ_{gy}^2), the tree × year (σ_{ty}^2), and among harvest dates within tree and year (residual variance) (σ_{e}^2) (Table 3). The σ_{e}^2 contains the error variance of the regression coefficient. The error variance is estimated as

$$\sigma_b^2 = \frac{\sum_{i=1}^{g}\sum_{j=1}^{t}\sum_{k=1}^{y}\sum_{l=1}^{h}\left[\left\{\sigma_{ijkl}^2/(Sx)_{ijkl}\right\}(m_{ijkl}-2)\right]}{\sum_{i=1}^{g}\sum_{j=1}^{t}\sum_{k=1}^{y}\sum_{l=1}^{h}(m_{ijkl}-2)}, \tag{3}$$

where σ_{b}^2 is the weighted average of the error variance of the regression coefficient; σ_{ijkl}^2 the regression residual variance of the *l*th harvest date in the *j*th tree of the *i*th genotype in the *k*th year; $(Sx)_{ijkl}$ is referred to above; $\sigma_{ijkl}^2/(Sx)_{ijkl}$ the error variance of the regression coefficient of the *l*th harvest date in the *j*th tree of the *i*th genotype in the *k*th year; m_{ijkl} is referred to above; and $(m_{ijkl} - 2)$ the degree of freedom of the σ_{ijkl}^2. True harvest variation (σ_{h}^2) can be estimated by subtracting σ_{b}^2 from the σ_{e}^2 (Table 4).

Optimum allocation

The variance of a mean of the regression coefficients in the *i*th genotype can be expressed as

$$\sigma_{\bar{b}}^2 = \frac{\sum_{j=1}^{t}\sum_{k=1}^{y}\sum_{l=1}^{h}\left\{\sigma_{ijkl}^2/(Sx)_{ijkl}\right\}}{(tyh)^2}, \tag{4}$$

where *y* is the number of yearly replications; *t* the number of tree replications; and *h* the number of harvest replications. Then, the environmental variance of a genotype mean (σ_{E}^2) can be expressed as

$$\sigma_{\mathrm{E}}^2 = \frac{\sigma_{\mathrm{gy}}^2}{y} + \frac{\sigma_{\mathrm{t}}^2}{t} + \frac{\sigma_{\mathrm{ty}}^2}{ty} + \frac{\sigma_{\mathrm{h}}^2}{tyh} + \sigma_{\bar{b}}^2 \tag{5}$$

When the regression residual variances σ_{ijkl}^2 are assumed to be equal to σ^2 and the number of sampling times for measuring firmness during storage and the number of fruit for each measurement are the same ($(Sx)_{ijkl} = Sx$) for all regressions, Eq. (4) becomes $\sigma_{\bar{b}}^2 = \sigma^2/(Sx)tyh$. Moreover, the sum of squares of an independent variable (*Sx*) can be expressed in this study condition as

$$Sx = \sum_{i=1}^{N}(x_i - \bar{x})^2 = fd^2C;$$

$$C = \left[\sum_{i=1}^{n}(i-1)^2 - \frac{\left\{\sum_{i=1}^{n}(i-1)\right\}^2}{n}\right], \tag{6}$$

where *f* is the number of fruits per firmness measurement; *d* the interval of firmness measurement (5 days); and *n* the number of sampling times for firmness measurement during storage. Consequently, the environmental variance of a genotype mean can be rewritten as

$$\sigma_{\mathrm{E}}^2 = \frac{\sigma_{\mathrm{gy}}^2}{y} + \frac{\sigma_{\mathrm{t}}^2}{t} + \frac{\sigma_{\mathrm{ty}}^2}{ty} + \frac{\sigma_{\mathrm{h}}^2}{tyh} + \frac{\sigma^2}{fd^2Ctyh}. \tag{7}$$

Each variance component in Eq. (7) has been obtained from Table 4, and the regression residual variances (σ^2) were estimated by Eq. (2). The optimum allocation can then be determined under a particular level of environmental variance by changing the number of years (y), trees (t), harvest dates (h), and sampling number of fruit (f) for each firmness measurement.

Results and discussion

The difference in the regression coefficient from harvest dates from one tree was significant at the 5% level (Table 2). The difference indicated that the softening rate was not equal in fruit harvested in subsequent harvest dates. For this study, fruits were harvested from a total of 30 trees of 13 genotypes in a year, and the experiment was repeated in 2 years (Table 1). When the ANOVA for the regressions obtained from three harvest dates was conducted for each tree separately, significant differences were detected in five trees of 30 trees in 2001, and in only one tree out of 30 trees in 2002. The genotype was different in all six trees, in which the softening rate was significantly different according to the harvest date. This means that the significant difference does not depend on the genotype.

Stow and Genge (2000) suggested that the picking date did not affect the softening rate during storage of the ‘Royal Gala’ apple, in which the difference in the picking date was 20 days, although the samples harvested later were always softer than the earlier ones. Moreover, in several studies, firmness at harvest was highly correlated with firmness after storage when fruit was harvested on several dates (Marmo et al., 1985; Knee & Smith, 1989). The correlation, in other words, indicated that the softening rate during storage was uniform regardless of the harvest date when the fruit was commercially mature. On the other hand, Ingle and Morris (1989) observed that a significant positive correlation coefficient was evident between firmness at harvest and changes in firmness during storage at 20 and 0 °C in the ‘Rome’ apple. Ingle and D’Souza (1989) also observed the same correlation in the ‘Red Delicious’ apple stored at 0 °C. Johnston et al. (2002) observed that fruit harvested later began to soften sooner than fruit harvested earlier although the rate of rapid softening did not differ according to the harvest dates in the ‘Royal Gala’ and ‘Cox Orange Pippin’ apples.

Table 2. Analysis of variance for regression among harvest and test for the significance of differences in the regression coefficient

Source of variation	*df*	Mean squares
Among regression coefficients on each harvest date	120	36.981*
Regression residual	2476	28.148

*Significant at $P < 0.05$, using F-test.

To determine the cause of the difference in the softening rate according to the harvest date, the relationship between the variance of fruit firmness according to the harvest date and the variance in the softening rate according to the harvest date was calculated for all entries. In addition, the relationship between the variance of SSC according to the harvest date and the softening rate was calculated. These calculations were necessary because of the probability that genotypes with a large difference in the physiological stage of maturity of fruit harvested 2 weeks after the first harvest date had a tendency to have large differences in softening rates. The results were that the relationships were low ($r = 0.2019$, 0.0887, respectively). Moreover, there was not a tendency for the fruit to soften faster or more slowly when the fruit was harvested later (data not shown). Therefore, the significant difference in the softening rate according to the harvest date, which reached the 5% level, could not have been induced by some particular genotype, or by the large difference in the physiological stage of maturity.

The length of the harvest period depends on the genotypes, and the harvest period may last a few weeks. Therefore, an ANOVA was conducted using the regression coefficient by defining the variance in the regression coefficient among three harvest dates at weekly intervals as the error variance to determine the significance of the effects of tree, year, and its interactions on the softening rate. The result of the ANOVA showed that the effects of genotype and year were highly significant at $p < 0.01$ (Table 3). On the other hand, the

Table 3. Analysis of variance for the regression coefficient of fruit softening during storage using 13 genotypes with 2 or 3 trees per genotype for 2 years

Source of variation	*df*	MS	Expected mean squares
Genotype	12	5.560**	$\sigma_e^2 + 3\sigma_{ty}^2 + 6\sigma_t^2 + 6.9\sigma_{gy}^2 + 13.8\sigma_g^2$
Year	1	8.202**	$\sigma_e^2 + 3\sigma_{ty}^2 + 7.2\sigma_{gy}^2 + 90\sigma_y^2$
Genotype × year	12	0.364^{NS}	$\sigma_e^2 + 3\sigma_{ty}^2 + 6.9\sigma_{gy}^2$
Among trees within genotype	17	0.249^{NS}	$\sigma_e^2 + 3\sigma_{ty}^2 + 6\sigma_t^2$
Year × tree	17	0.243*	$\sigma_e^2 + 3\sigma_{ty}^2$
Among harvest within tree and year	120	0.140	σ_e^2

NS: Non-significant.

*,** indicates significance at $P < 0.05$ and $P < 0.01$, respectively.

Table 4. Estimates of variance components and their percentage to the total variance obtained from the analysis of variance for the regression coefficient of fruit softening during storage using 13 genotypes with 2 or 3 trees per genotype for 2 years

Variance components		Estimates	
Genotype	σ_g^2	0.376	57.3%
Year	σ_y^2	0.087	13.3%
Genotype × year	σ_{gy}^2	0.018	2.7%
Among tree within genotype	σ_t^2	0.001	0.1%
Year × tree	σ_{ty}^2	0.034	5.2%
Among harvest	σ_e^2	0.140	21.4%
True harvest variance	σ_h^2	0.037	5.7%
Error variance of regression coefficient	$\sigma_{\bar{b}}^2$	0.103	15.7%
Total		0.656	100%

Table 5. Softening rates of 13 apple cultivars and selection averaged from three harvest dates from two or three trees of each genotype for 2 years

Genotype	Softening rate (*N*/day)	Environmental deviation[a] ($\sqrt{\sigma_E^2}$)
Silken	2.850	0.186
Himekami	2.546	0.175
Akane	2.464	0.178
Morioka57	2.445	0.207
Kotaro	2.091	0.192
Sannsa	2.083	0.158
Jonathan	1.929	0.191
Orin	1.927	0.152
Ralls Janet	1.847	0.169
Golden Delicious	1.834	0.173
Santaro	1.478	0.169
Tsugaru	1.382	0.147
Fuji	0.498	0.135

[a] Calculated using Eq. (5).

effects of trees within genotype and genotype × year were nonsignificant.

The variance associated with genotype (σ_g^2) was the largest, namely, more than half of the total variance (Table 4). The variance among harvest dates was next, being 21% of the total variance. The variance among harvest dates, however, contained the error variance of the regression coefficient. The error variance of the regression coefficient was 75% of the variance among harvest dates (Table 4). Therefore, the environmental variance components associated with year (σ_{gy}^2, σ_{ty}^2), tree (σ_t^2), and harvest date (σ_h^2) were all very small though the variance associated with years (σ_y^2) was large (13% of total variance).

In apple breeding, using trees that are planted in the same year or grafted on the same rootstock for comparison of genotypes is not always possible (Durel et al., 1998; Hampson et al., 2000). For this study, therefore, trees of various ages and rootstocks were examined to determine how environmental variances contributed to the genotype means. Since the variance components associated with trees and other environmental factors were small despite the fact that trees of various types had been used, this method appears to be efficient for the comparison of genetic differences in softening by using regression coefficients. Although fruit of all genotypes except ‘Fuji’ softened within a storage period of approximately 10 days (Table 1), the softening rates of the genotypes varied from 1.38 to 2.85 (Table 5) and were significantly divided into six groups by calculating $\pm 1.96\sqrt{\sigma_E^2}$ as the 95% confidence interval.

The environmental variance (σ_E^2) can be reduced and, thus, the precision of estimates for the genotype mean of softening rate can be increased most efficiently by reducing the contributions of σ_{ty}^2, σ_h^2, and $\sigma_{\bar{b}}^2$, which were relatively large (Table 4). Since both the year and tree replications can equally reduce the contributions of the σ_{ty}^2, σ_h^2, and $\sigma_{\bar{b}}^2$ (Eqs. (4) and (5)), year and tree replications are more efficient than harvest replications or increasing the number of harvest fruit.

The cost for breeding depends on the space in the field and the speed of selection (Yamada et al., 1993). Tree replication reduces the number of genotypes that can be evaluated in a restricted breeding field, and the trees continue to occupy the field during yearly repeated evaluations. Thus, yearly repetitions or tree replications are costly and time-consuming, and a comparison between yearly repetitions and tree replications should be primarily considered (Sato et al., 2000). Yamada et al. (1993), however, indicated that, unless the variance associated with trees within a genotype (σ_t^2) is markedly greater than the variance associated with the genotype × year interaction (σ_{gy}^2), it is disadvantageous to increase the number of tree replications while sacrificing yearly repetitions in every breeding situation in which seedlings cannot bear fruit in the first year of planting. In this study, the year replications appeared to be more efficient for estimating the genotype mean of the softening rate than the tree replications because the $\sigma_t{}^2$ was considerably smaller than the σ_{gy}^2.

The genotype differences in the softening rate among 13 cultivars and selections were evaluated with about two trees from each genotype and two annual repetitions. Since the tree replications are disadvantageous in a breeding situation, alternative allocations of the repetitions were then examined as a means of detecting significant differences in the softening rate among genotypes with the same accuracy as in the current study. Equation (7) was solved for f under various replications of years, trees, and harvest times. The standard environmental variance ($\sqrt{\sigma_E^2}$) was defined as 0.2, which was about the highest value obtained in this study. *C* in Eq. (6) was calculated by substituting three for *n*; this means that the firmness measurement is carried out three times at 5-day intervals from the harvest date until 10 days after storage, since fruit from almost all of the genotypes in this study soften within approximately 10 days of storage.

The number of fruit necessary for firmness measurements to obtain 0.2 in standard environmental variance is given in Table 6. The results demonstrate that 14 fruits are necessary when fruit are harvested at three times from a single tree of each genotype for 2 years, while 0.2 in standard environmental deviation cannot be obtained when fruit are harvested at one time. When fruits are to be harvested at one time from a single tree, the evaluation of softening needs to be continued for 3 years, and 21 fruits are necessary for each measurement for each year. On the other hand, only six fruits are necessary when fruits are harvested twice under the same conditions. Therefore, although harvest on several dates is likely to be more costly, at least two harvests are necessary to determine a genotype mean for the softening rate.

Estimations of the variance component generally depend on the experimental populations and could vary. However, the environmental variance components estimated in this study were all small, although genotypes of relatively wide variation were used with trees under various conditions. Therefore, this method for evaluating softening is stable and may be useful for other breeding locations and apple populations.

Table 6. Comparisons of the number of fruit for firmness measurements for some alternative replications of years, trees, and harvest dates when the standard environmental deviation ($\sqrt{\sigma_E^2}$) was defined as 0.2 for the genotypes in which the fruit softened after 10 days of storage

		No. of fruit for measurement		
		harvest replications		
No. of year replications	No. of tree replications	1	2	3
2	1	–	37.8	13.7
	2	11.0	4.0	2.5
3	1	20.4	6.1	3.6
	2	4.3	1.9	1.2

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