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Phenotypic Selection to Avoid Discarding Target Genotypes for Four Fruit Traits Based on Environmental Variances in a Pineapple Cross-seedling Population

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Abstract. **Early ripening [earlier than 1 Aug during fruit harvest time (FHT)], large fruit weight (FW; >1000 g), high sugar content [>17% soluble solids content (SSC)], and low acidity in fruit juice (<0.7%) are important breeding targets of pineapple for table fresh fruit use in Japan. We investigated the efficiency of primary selection based on the four fruit traits using 129 first-fruiting F_1 offspring population of 'Yugafu' × 'Yonekura' without replicates. Separately, environmental variances were estimated by an analysis of variance using evaluation data from 50 or 49 offspring in three replicates and two-year repeats. The phenotypic distribution in the 129 F_1 population approached a normal distribution ($P > 0.05$). The genotypic distribution was obtained as a normal distribution with the population mean as the mean and genotypic variance obtained by subtracting the environmental variance from the phenotypic variance. The target genotypes were estimated at 14.4%, 58.7%, 5.0%, and 50.0% of the F_1 population for FHT, FW, SSC, and acidity, respectively. Critical phenotypic values were established as the upper (FHT and acidity) and lower (FW and SSC) limits of the critical genotypic values at the 95% probability level. The phenotypic selection was made based on the critical phenotypic value, resulting in 45.0%, 88.4%, 27.1%, and 79.1% of the offspring selected for FHT, FW, SSC, and acidity, respectively, and 12.4% simultaneously for all four fruit traits. The results showed that the phenotypic primary selection reduced the population size to 12.4%, avoiding the discarding of target genotypes with a low risk. If breeders intend to further reduce the population size, then increasing the number of traits subject to primary selection would be effective.**

Pineapple [*Ananas comosus* (L.) Merr.] is an important tropical fruit crop, the global production of which was estimated at ∼29 million tons (mt), which is twice that of papaya production (14 mt), 23% of banana production (125 mt), 31% of apple production (93 mt), and almost comparable to that of peach production (25 mt) and pear production (26 mt) in 2021 (FAOSTAT 2023). Its domestic production in Japan, which has a mostly temperate climate, is restricted to the southern and western islands of mainly Okinawa Prefecture and reached 7278 tons in 2021 (FAOSTAT 2023). Pineapple adapts well to the subtropical climate of these areas and ranks first in production among fruit crops in Okinawa.

In the twentieth century, the leading pineapple variety in the world had been 'Smooth Cayenne'. The Pineapple Research Institute in Hawaii developed new cultivars through crossbreeding, including 'MD2', which is currently a leading cultivar worldwide (Ogata et al. 2016; Williams and Fleisch 1993). Although the Pineapple Research Institute experiment station was closed in 1975, crossbreeding has been performed in several countries, including Taiwan, Malaysia, Australia, Japan, South Africa, India, China, Cote d'Ivoire, Brazil, and other countries (Li et al. 2022; Ogata et al. 2016). With the fresh expansion of the pineapple fruit market, fruit quality has become an increasingly important feature, and the objectives of most pineapple breeding programs have gradually changed from high-yield and easy-processing, and mainly canning to high-quality fresh fruit (Li et al. 2022).

Pineapple is a perennial herbaceous crop that is propagated vegetatively using suckers, shoots, slips, and crowns (Samson 1986). The cultivated pineapple plant does not produce seeds because of its strong self-incompatibility (Kudo and Koga 1981), resulting in the absence of inbred lines. Many different genotypes of the F_1 offspring arise from crosses among cultivars/selections. A superior clone is selected from F_1 offspring from many crosses, propagated vegetatively, and released as a new cultivar. The probability of obtaining a new cultivar among F_1 offspring is generally very low, resulting in many crosses and the raising of many F_1 offspring plants for several years. Therefore, the cross-breeding procedure is similar to that used for woody fruit crops (Ogata et al. 2016). In Japan, pineapple crossbreeding has been conducted for the fresh fruit market in the Nago Branch of the Okinawa Prefectural Agricultural Research Center (OPARC-Nago; 26°62′N, 127°98′E); subsequently, eight new cultivars were released, and all of them have a higher sugar content than 'Smooth Cayenne' (Ogata et al. 2016).

In the pineapple cross-breeding program at OPARC-Nago, 4000 pineapple cross-seedlings are normally obtained from 20 to 30 artificial hybridizations every year, planted in a field without plant replication, and subjected to primary selection. A plant bears one fruit from natural flowering in the summer 2 years after field planting. Primary selection focuses on evaluating some commercially important fruit traits [fruit harvest time (FHT), fruit weight (FW), high sweetness as the soluble solids content (SSC), and low acidity]. During primary selection, approximately 50 promising individuals (cross-seedlings) are normally selected from the 4000 cross-seedlings. Selected cross-seedlings are vegetatively propagated, planted again in a field with approximately five replicates per cross-seedling (genotype), and the fruit traits, yield, disease and pest resistance, ease of vegetative propagation, and physiological disorders in the secondary selection are evaluated. During the pineapple selection process, the F_1 offspring are vegetatively propagated to evaluate their genetic property more precisely.

An early objective in plant breeding programs is to decrease the population size after primary selection and avoid discarding target genotypes for the four primary selection traits. However, these fruit traits are quantitative and usually fluctuate because of environmental factors. Estimation of environmental variance requires evaluation repetitions and plant replicates. In a pineapple F_1 cross-seedling, a plant bears one fruit without plant replicates; therefore, the fruit trait cannot be repeatedly evaluated, resulting in the breeder not being able to estimate environmental variances for the fruit traits. To overcome this restriction, we used a seedling population from the cross

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'Yugafu' × 'Yonekura' that was vegetatively propagated, estimated environmental variances, and determined the selection efficiency in primary selection. Lira et al. (2021) reported selection in a pineapple offspring population by estimating genotypic values as accurately as possible with fruit trait evaluations in replications. In contrast, in this study, we investigated primary practical selection with environmental bias without replication and selection efficiency based on the loss of target genotypes by selection. To the best knowledge of the authors, no studies have reported these aspects regarding the breeding of pineapples.

The objectives of the present study using this offspring population were to estimate environmental variances, estimate the proportion of target genotypes that existed in the offspring population, establish a critical phenotypic value in primary selection and minimize the loss of F_1 genotypes that exceeded the critical genotypic value (target genotypes) at the 95% probability level for each of the four traits, and estimate the proportion of individuals selected above or below the critical phenotypic values in the offspring population (population size after primary selection), which indicates selection efficiency.

Materials and Methods

Environmental variance estimation using 50 (49) F_1 offspring, with three planting blocks for 2 years. A cross of 'Yugafu' × 'Yonekura' was made in 2010, and cross-seedlings were obtained at OPARC-Nago. The cross is one of the crosses in a practical breeding program. Yugafu is a cultivar recently released by OPARC-Nago and an F_1 from 'Cream Pineapple' × HI101 (Shoda et al. 2012). Cream Pineapple and HI101 are a cultivar and a breeding line, respectively, introduced from the United States to Okinawa (Shoda et al. 2012). Yonekura is a cultivar introduced from Hawaii; however, the details of its origin are unclear. Nashima et al. (2020) identified 'Yonekura' as a clone of 'MD2' using simple sequence repeat (SSR) markers. In the cross, 'Yugafu' was used as a cross-parent with good fruit-eating quality, large fruit size, and high adaptability for the Okinawa climate. 'Yonekura' was used as a possible source of long shelf life, fruitlet core rot tolerance (Yamashiro et al. 2019), and yellow flesh color. These cross-seedlings were planted in a pineapple selection field at OPARC-Nago at a density of 40,000 plants/ha, which is the normal planting density for commercial pineapple production in Okinawa. Each plant bore one fruit in Summer 2013, that was harvested at its respective ripening time and evaluated to determine the FHT, FW, SSC, and acidity. The FHT was determined based on 50% yellow coloration and dull tapping sound in a fruit. The FHT was arbitrarily expressed as the number of days after 1 Jun. The SSC and acidity were measured by a sugar and acid content analyzer (NH-2000; HORIBA Ltd., Kyoto, Japan) based on the refractometric method (Brix%) and the conductometric method (acidity %), respectively. Its measured value of acidity was highly correlated with the total acid content determined by high-performance liquid chromatography (HPLC) using 430 fruits from 15 cultivars of pineapple (r = 0.93) (Takeuchi et al., unpublished data).

Cross-seedling plants were propagated vegetatively using the shoots (Samson 1986). Three clones derived from a cross-seedling (genotype) were planted separately in three blocks of the pineapple selection field. During Summer 2015, these plants bore fruits that were evaluated to determine the four fruit traits in the same way as performed for those harvested in 2013. Again, these plants were vegetatively propagated, and three clones per genotype were planted in another three blocks of the pineapple selection field; subsequently, fruits were harvested and evaluated during Summer 2017 in the same way as performed during 2015.

The fruit trait data evaluated in 2015 and 2017 were subjected to an analysis of variance (ANOVA) using the model shown in Eq. [1]. Before the analysis, the SSC and acidity data were log-transformed and denoted as logSSC and logAcidity, respectively, because the original data showed a correlation between the mean and *SD* (Snedecor and Cochran 1972). The model for the evaluated phenotypic value (P_{ijk}) was as follows:

$$P_{ijk} = \mu + y_i + g_k + (gy_{ik}) + b_{ij} + r_{ijk} \quad [1]$$

where μ is a constant (overall mean), y_i is the effect of the ith year, g_k is the effect of the kth genotype, $(gy)_{ik}$ is the interaction between the ith year and the kth genotype, b_{ij} is the effect of the jth block within the ith year, and r_{ijk} is the residual effect of the jth block of the ith year in the kth genotype. The numbers of genotypes were 50 for FHT and FW and 49 for SSC and acidity, whereas the number of years and blocks were two and three, respectively.

The ANOVA provided the genetic variance (σ_g^2) and environmental variance components, with the latter consisting of the year variance (σ_y^2), block variance within years (σ_b^2), genotype × year (σ_{gy}^2), and residual variance (σ^2). For all four fruit traits, the distribution of the residual estimates approximated a normal distribution (insignificant at the 5% level in the Kolmogorov Smirnov one-sample test), indicating that the ANOVA was applicable (Campbell 1974). The total variance was calculated as the sum of those variance estimates ($\sigma_g^2+\sigma_y^2+\sigma_b^2+\sigma_{gy}^2+\sigma^2$). The total environmental variance was calculated as $\sigma_y^2+\sigma_b^2+\sigma_{gy}^2+\sigma^2$.

Critical genotypic and phenotypic values of the selection and phenotypic selection for the 129 F_1 offspring population. Breeders have to make practical selections for the first fruiting F_1 offspring plant population; therefore, we used the four fruit trait evaluation data of 129 F_1 offspring population of 'Yugafu' × 'Yonekura' in 2013, which consisted of first fruiting F_1 seedlings, and made an experimental primary selection for the data. Based on the breeding objectives, the critical genotypic value for selecting the target genotypes was set as <61 d (earlier than 1 Aug) for FHT, >1000 g for FW, >17 Brix% for SSC, and <0.7% for acidity for the 129 F_1 offspring population in 2013. The genotypic values were assumed to be distributed normally (genetic distribution) with the population mean and genetic variance of the 129 F_1 offspring population. The 129 F_1 offspring data were obtained for a year (2013) and a block of the pineapple selection field. The genetic variance was estimated by subtracting the environmental variance within a year and a block ($\sigma_{gy}^2 + \sigma^2$) from the variance of the data evaluated in 2013 (phenotypic variance). The probability below the critical genotypic value in the genetic distribution obtained was calculated for FHT and logAcidity using the NORM.DIST function of Microsoft Excel 2019, and it was assumed to be the proportion of the target genotypes in the population. In the same way, the probability exceeding the critical genotypic value was calculated and assumed to be the proportion of target genotypes for FW and logSSC.

The phenotypic value of a genotype with the critical genotypic value was assumed to be normally distributed with the critical genotypic value as the mean and the environmental variance as the variance with one plant evaluation in 2013, which was the environmental variance (σ_E^2) within a year and a block estimated as $\sigma_{gy}^2 + \sigma^2$. The critical genotypic values (G_c) were 61 d for FHT and 0.7% for acidity (−0.1549 for logAcidity), respectively. The upper limit of the 95% probability level calculated as $G_c+1.64485\sigma_E$ using Microsoft Excel 2019 for FHT and logAcidity was assumed to be the critical phenotypic value for selection. It was 82.9 d for FHT and −0.0577 for logAcidity (0.876% for acidity), respectively. The proportion of phenotypic values below the critical phenotypic value for FHT and logAcidity was assumed to be the proportion of the F_1 offspring selected from the F_1 offspring population in 2013.

In the same way, the critical genotypic values were 1000 g for FW and 17% for SSC (1.230 for logSSC), respectively. The lower limit of the 95% probability level for FW and logSSC as $G_c-1.64485\sigma_E$ was assumed to be the critical phenotypic value for selection. It was 584.2 g for FW and 1.181 for logSSC (15.2% for SSC), respectively. The proportion of phenotypic values above the critical phenotypic values for FW and logSSC was assumed to be the proportion of the F_1 offspring selected from the F_1 offspring population in 2013.

Results

Environmental variance estimation using 50 (49) F_1 offspring with three planting blocks for 2 years. The ANOVA results showed that the effects of genotype were highly significant ($P < 0.01$) for all four fruit traits (Table 1). The degrees of freedom were 49 for FHT and FW and 48 for logSSC and logAcidity. The percentages of σ_g^2 to the total variance (broad sense heritability) were

Table 1. Analysis of variance of the data of four pineapple fruit traits using three blocks and 2 years (2015 and 2017).

Factor	Degree of freedom[i]	Mean square FHT	FW ($\times 10^2$)	SSC (log-transformed) ($\times 10^{-5}$)	Acidity (log-transformed) ($\times 10^{-5}$)	Expectation of the mean square[i]
Year	1	358.6^{NS}	4015.8^{NS}	135.6^{NS}	4558.9^{NS}	$\sigma^2+3\sigma_{gy}{}^2+50$ (49) $\sigma_b{}^2+150$ (147) $\sigma_y{}^2$
Genotype	49 (48)	1799.3**	4402.8**	1190.9**	2276.1**	$\sigma^2+3\sigma_{gy}{}^2+6\sigma_g{}^2$
Genotype × year	49 (48)	209.1^{NS}	743.9^{NS}	122.4**	489.5**	$\sigma^2+3\sigma_{gy}{}^2$
Among blocks within year	4	489.4*	731.8^{NS}	29.9^{NS}	1734.7**	σ^2+50 (49)$\sigma_b{}^2$
Error	196 (192)	162.1	586.6	72.9	279.0	σ^2

[i] Numerical values in parentheses indicate SSC and acidity.
NS, *, ** Not significant or significant at $P < 0.05$ or 0.01, respectively.
FHT = fruit harvest time; FW = fruit weight; SSC = soluble solids content.

estimated at 59.0%, 47.9%, 66.5%, and 64.0% for FHT, FW, logSSC, and logAcidity, respectively (Table 2).

The year effect was not significant for the four fruit traits (Table 1), and the percentage of $\sigma_y{}^2$ to the total variance was negligible for all fruit traits (Table 2). The results indicated that the measured values of all offspring genotypes rarely varied yearly in parallel for the four fruit traits during 2015 and 2017.

The genotype × year was not significant for FHT and FW, but it was significant for logSSC and logAcidity (Table 1). The percentages of $\sigma_{gy}{}^2$ to the total variance were estimated at 3.5%, 4.1%, 6.2%, and 6.6% for FHT, FW, logSSC, and logAcidity, respectively (Table 2). The results indicated a small degree of yearly fluctuation; namely, some genotypes had larger values during one year than during another year, and other genotypes had smaller values.

The effect of the blocks was significant for FHT and logAcidity, but it was not significant for FW and logSSC (Table 1). However, the percentages of $\sigma_b{}^2$ to the total variance were estimated at 1.5%, 0.2%, 0.0%, and 2.8% for FHT, FW, logSSC, and logAcidity, respectively; therefore, they were negligible for the four fruit traits (Table 2). These results suggested that environmental conditions, including soil and planting conditions, were uniform across the blocks in the entire field at OPARC-Nago.

The residual variance (σ^2) was the largest environmental component of the four fruit traits (Table 2). The percentages of σ^2 to the total variance were 36.1%, 46.1%, 27.2%, and 26.3% for FHT, FW, logSSC, and logAcidity, respectively. They were 88.0%, 88.5%, 81.2%, and 73.2% of the total environmental variance for FHT, FW, logSSC, and logAcidity, respectively. This indicated that environmental variations in the measured data were largely caused by variations among plants within a block and year.

Estimation of the percentage of the target genotype offspring in the 129 F_1 offspring population. Deviations in the distribution of the 129 measured values in 2013 (phenotypic values) from the normal distribution were not significant ($P > 0.05$) according to the Kolmogorov-Smirnov one-sample test, and the phenotypic value distribution approached a normal distribution for all fruit traits (Fig. 1). The distribution of the genotypic values was assumed to be the normal distribution with the mean value of the 129 measured values as the mean and the genetic variance as variance. The percentages of cumulative probability below the critical genotypic value [61 d FHT and −0.155 logAcidity (0.7% Acidity)] in the normal distribution were 14.4% for FHT and 50.0% for logAcidity (Table 3). The percentages of cumulative probability above the critical genotypic value [1000 g FW and 1.230 logSSC (17 Brix%)] in the normal distribution were 58.7% for FW and 5.0% for logSSC. Each of the four percentages indicated the estimated percentage of the target genotypes existing in the F_1 offspring population from 'Yugafu' × 'Yonekura' for each fruit trait. The product of the four proportions (0.144 × 0.500 × 0.587 × 0.05) was 0.002 (that is, 0.2%) (Table 3), which was an estimate of the percentage of the target genotype combining the desirable genotypic values over the four fruit traits in the F_1 population, with no genetic correlations among the fruit traits.

Phenotypic correlation among the four fruit traits. The phenotypic correlation (Pearson's correlation coefficient r) among the four fruit traits for the measured values of the 129 F_1 population in 2013 are listed in Table 4. The correlation was significant between FHT and FW, FHT and logAcidity, FW and logSSC, and FW and logAcidity, but not between FHT and logSSC, and between logSSC and logAcidity. These results suggested that late-ripening individuals were likely to have small fruits with higher acidity, and that individuals with large fruits were likely to have low SSC and acidity.

Phenotypic selection using the 129 F_1 offspring population: A critical phenotypic value in selection and the proportion of individuals selected above/below the critical phenotypic values in the offspring population. The critical phenotypic values during selection were set as 82.9 d for FHT, 584.2 g for FW, 1.181 (logSSC) (which was 15.2 Brix%) for SSC, and −0.058 (logAcidity) (which was 0.876%) for acidity (Table 3). The numbers of F_1 offspring individuals below the critical phenotypic value (82.9 d FHT and 0.876 acidity) in the 129 F_1 offspring were 58 individuals for FHT and 102 individuals for acidity, comprising 45.0% and 79.1% of the total 129 F_1 offspring, respectively (Table 3). The numbers of F_1 offspring above the critical phenotypic value (584.2 g for FW and 15.2 Brix% for SSC) in the 129 F_1 offspring were 114 individuals for FW and 35 individuals for SSC, comprising 88.4% and 27.1% of the total 129 F_1 offspring individuals, respectively. The combined number of F_1 offspring above/below the critical values for the four fruit traits was 16 individuals, comprising 12.4% of the 129 F_1 offspring individuals. The product of the four proportions (0.450 × 0.791 × 0.884 × 0.271) was 0.085 (that is, 8.5%) (Table 3), which was an estimate of the percentage of offspring individuals above/

Table 2. Variance estimates of pineapple trials with 50 (49) genotypes using three blocks and two years (2015 and 2017).[i]

Variance component	FHT Variance estimate	FHT Percentage to the total variance (%)	FW Variance estimate ($\times 10^2$)	FW Percentage to the total variance (%)	SSC (log-transformed) Variance estimate ($\times 10^{-5}$)	SSC (log-transformed) Percentage to the total variance (%)	Acidity (log-transformed) Variance estimate ($\times 10^{-5}$)	Acidity (log-transformed) Percentage to the total variance (%)
$\sigma_g{}^2$	265.0	59.0	609.8	47.9	178.1	66.5	678.2	64.0
$\sigma_y{}^2$	0.0	0.0	20.8	1.6	0.4	0.1	2.3	0.2
$\sigma_{gy}{}^2$	15.7	3.5	52.4	4.1	16.5	6.2	70.2	6.6
$\sigma_b{}^2$	6.5	1.5	2.9	0.2	0.0	0.0	29.7	2.8
σ^2	162.1	36.1	586.6	46.1	72.9	27.2	279.0	26.3

[i] Numerical values in parentheses indicate SSC and acidity.
FHT = fruit harvest time; FW = fruit weight; SSC = soluble solids content.

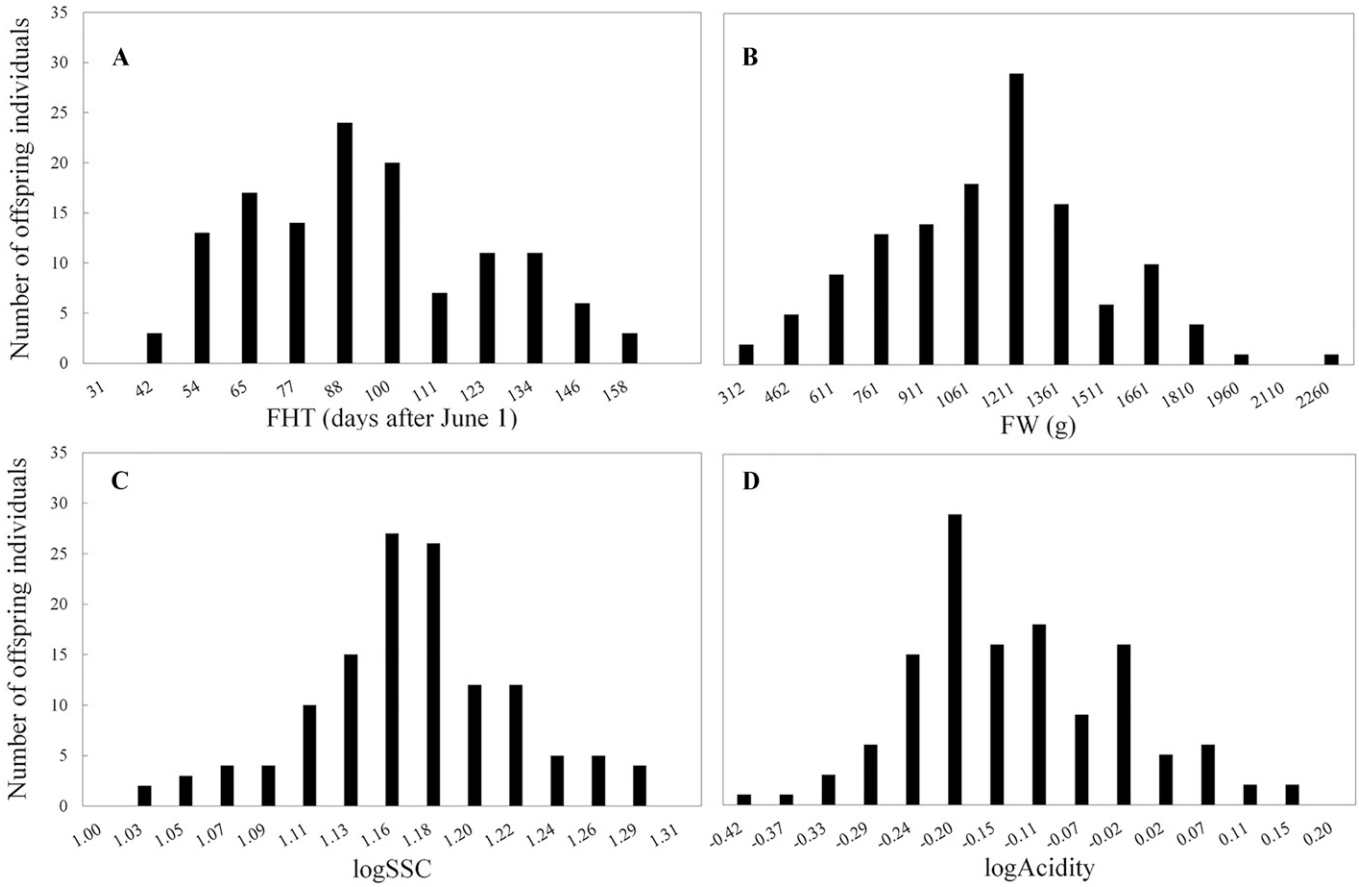

Fig. 1. Histogram of the measured phenotypic values of the 129 F1 offspring population yielded from 'Yugafu' × 'Yonekura' for fruit harvest time (**A**), fruit weight (**B**), logSSC (soluble solids content) (**C**), and logAcidity (**D**). The numerical value in the horizontal axis indicates an upper limit in each class.

below the critical phenotypic values of the four fruit traits in the F_1 population, with no correlation among the fruit traits and deviated slightly from 12.4%.

The distribution of the phenotypic values was assumed to be normal, with the mean value of the 129 measured values as the mean and the phenotypic variance as the variance. The percentages of cumulative probability below the critical phenotypic value [82.9 d FHT and −0.058 logAcidity (0.876% for acidity)] in the normal distribution were 42.7% for FHT and 81.2% for logAcidity (Table 3). The percentages of cumulative probability above the critical phenotypic value [584.2 g FW and 1.181 logSSC (15.2 Brix%)] in the normal distribution were 89.8% for FW and 32.1% for logSSC. The product of the four proportions (0.427 × 0.812 × 0.898 × 0.321) was 0.100 (that is, 10.0%).

Discussion

Basis of assuming normal distribution. Quantitative traits are controlled by several gene loci and are likely to fluctuate because of environmental effects caused by many factors, including climate conditions, soil conditions, and cultural practices (Ukai 2002). Using statistics, the sample mean tends toward a normal distribution even if the original variables themselves are not normally distributed (central limit theorem) (Snedecor and Cochran 1972). Both genetic and environmental effects can be regarded as samples of genetic effects at many gene loci and environmental effects caused by many factors; therefore, measurable phenotypic values approach a normal distribution in many cases, and approach a normal distribution after some transformation in some cases. In the present study, the phenotypic values of the 129 F_1 offspring approached a normal

Table 3. Critical genotypic and phenotypic values and percentages of offspring individuals selected for four fruit traits.

Fruit traits	Critical genotypic value of selection	Estimated percentage of target genotypes of the 129 offspring population (%)	Critical phenotypic value of selection	Number of selected offspring individuals from the 129 offspring population	Percentage of theoretically selected offspring individuals based on normal distribution of offspring phenotypic values (%) (P_2)
FHT	<61 d	14.4	<82.9 d	58 (45.0)[1]	42.7
FW	>1000 g	58.7	>584.2 g	114 (88.4)	89.8
SSC	>17%	5.0	>15.2%	35 (27.1)	32.1
Acidity	<0.7%	50.0	<0.876%	102 (79.1)	81.2
Total (of the four fruit traits)				16 (12.4)	
Product of the proportions of selected offspring for each of the four fruit traits (%)		0.2		8.5	10.0

[1] Numerical values in parentheses indicate the percentage of the 129 offspring individuals (P_1).

FHT = fruit harvest time; FW = fruit weight; SSC = soluble solids content.

Table 4. Phenotypic Pearson correlation coefficients among the four fruit traits of the 129 F_1 offspring population evaluated in 2013.

	FW	logSSC	logAcidity
FHT	−0.42*	-0.07^{NS}	0.57*
FW		−0.33*	−0.37*
logSSC			-0.01^{NS}

NS, * Not significant at $P = 0.05$ or significant at $P < 0.01$, respectively.
FHT = fruit harvest time; FW = fruit weight; SSC = soluble solids content.

distribution for all four fruit traits (Fig. 1). A normal distribution is unmistakably defined by only two variables: mean and variance. Therefore, based on normal distribution, simple analyses are available using the mean and variance.

Proportion of the target genotypes in the F_1 population for the four fruit traits and population size in the primary selection. Assuming a normal distribution of genotypic values in the F_1 offspring population, the proportion of target genotypes that existed in the population was estimated for each of the four fruit traits, and the percentage of the target genotype combining desirable genotypic values of the four fruit traits was estimated at 0.2% in the case of no genetic correlations among the fruit traits. This suggested that the target genotypes were estimated to exist in the population at a low percentage. However, significant positive or negative (but not high) phenotypic correlations among the four fruit traits were observed (Table 4), indicating that precisely estimating the percentage of the target genotype combining desirable genotypic values of the four fruit traits is difficult because of the offset effects of complicated correlations among traits.

The proportion of the target genotype combining desirable genotypic values of the four fruit traits (G) is important for breeders because it decides the population size that is required to be raised by crossing. For example, when breeders intend to obtain 20 target genotypes for the four fruit traits after primary selection, they should raise 20/G offspring seedlings before primary selection. When G = 0.002 (0.2%) is adopted, breeders must raise 10,000 offspring seedlings before primary selection. The rate of 0.2% is quite low for breeders, indicating that breeding is not easy. This estimation is not accurate because of the complicated correlations among the four traits, but it is practically important as an indicator of the F_1 population size that breeders should produce because there are no other indicators. The proportion of the F_1 offspring with the combined above/below critical values of the four fruit traits (12.4%) did not deviate much from the product of the four proportions (0.450 × 0.791 × 0.884 × 0.271) (8.5%), with no phenotypic correlation among the fruit traits (Table 3). Therefore, the 0.2% is recommended to be used in the breeding.

Phenotypic selection using 129 F_1 offspring population. The phenotypic value of a genotype with a critical genotypic value was assumed to be distributed normally, with the critical genotypic value as the mean and the environmental variance (σ_E^2) as the variance. The upper/lower limit of the 95% probability level in the distribution was set as the critical phenotypic value for the selection of each of the four fruit traits. The percentages of offspring individuals above/below the critical phenotypic value in the selection (P_1), which were obtained by counting, were nearly the same as the theoretical percentages based on the normal distribution of phenotypic values (P_2) for each of the four fruit traits (Table 3). P_2 was obtained as the cumulative probability above/below the phenotypic critical value of the normal distribution with the population mean as the mean and the phenotypic variance as the variance of the 129 F_1 offspring. The small difference between the P_1 and P_2 values suggested that the phenotypic distributions approached normal distributions well.

The population size after primary selection for the four fruit traits decreased to 12.4% of the initial population size, with a risk of 5% loss of the target offspring genotypes for a fruit trait. The total risk of the loss of the target offspring genotypes may be estimated as 18.5% for the four fruit traits ($1 - 0.95^4 = 0.185$) in the case of no genetic correlations among the four fruit traits.

Selection efficiency can be measured by the decrease in the population size under selection. Increased selection efficiency is inevitably accompanied by an increased risk of losing target offspring genotypes. In the present study, the critical phenotypic value was set as a risk of 5% loss for a fruit trait. However, the population size after primary selection can be further decreased in cases of increased risk (for instance, ≥10% loss for a trait), resulting in a smaller population size after primary selection. Breeders may not want a large secondary population because of increased breeding costs. Therefore, breeders should establish a balance between the risk of the loss of target offspring genotypes and population size after primary selection.

Selecting a considerably low percentage of offspring for a trait leads to a considerable decrease of the population size after primary selection. In the present study, during selection by only logSSC, the population size decreased to 27.1%. In contrast, the population size decreased to 88.4% during selection by FW. The results indicated that the population size decreased remarkably with selection by SSC and did not decrease significantly with selection by FW.

The greater the number of target traits, the smaller the population size after primary selection. With a simple calculation without any correlation among traits, the proportion of offspring individuals after selection with a combination of several traits is the product of the proportion of offspring selected for each trait. Adding more traits to the four fruit traits studied, such as disease resistance, fruit-keeping quality, ease of vegetative propagation, and physiological disorder, can decrease the population size. If breeders intend to decrease the secondary population size further, then it would be effective to add objective traits to primary selection.

Application of phenotypic primary selection to avoid discarding target genotypes with a low risk. The results of the present study revealed that primary selection based on the four fruit traits to avoid discarding the target genotypes effectively reduced the offspring population to 12.4%, indicating its selection efficiency.

So far, the selection theory for narrow sense heritability and genetic gain described comprehensively by Falconer (1960) aimed at crossbreeding annual allogamous crops and animals was applied to various perennial or woody fruit crops and has been reported by many studies (Abe et al. 1995; de Souza et al. 1998; Hansche 1983; Luby et al. 2002; Zeinanloo et al. 2009), including those of pineapple (Sanewski and De Faveri 2017). However, the theory is based on random mating populations and mass selection and states that the indicator of genetic improvement is the population mean over generations.

In contrast, F_1 offspring are vegetatively propagated and subjected to repeated selections (secondary and tertiary selection), but not over generations, to assess their genetic properties more precisely during practical pineapple selection. The indicator of genetic improvement during the selection process should not be the population mean; instead, it should be the population size after selection to avoid discarding the target genotypes. Therefore, the method used to quantify selection efficiency in the present study seems novel and practical because no studies have reported these aspects. This method may be applied to various woody fruit crop breeding programs that have selection procedures similar to those for pineapple breeding (Yamada 2011).

During OPARC-Nago pineapple crossbreeding, cultivars/selections have been developed from several different types of cultivars (Cayenne, Queen, Maipure, Spanish, and others), and breeding lines and foreign accessions have been used as sources of specific characteristics (e.g., early ripening, high sugar content, and low acidity) (Shoda et al. 2012). Recently, 'Yugafu', 'Cream Pineapple', and 'N67-10' (a vegetative selection from 'Hawaiian Smooth Cayenne') have been used as important cross-parents (Ogata et al. 2016; Shoda et al. 2012). In addition, more recently, their resultant cultivars/selections and 'MD2' have been used. Practical breeding populations with good fruit qualities are related to the population used in the present study. The present study showed the results of experimental primary selection for a pineapple offspring population that resulted from a cross. However, the method of quantifying selection efficiency may be applied to a large practical population consisting of offspring populations from several crosses. Environmental variance estimates and critical phenotypic selection values obtained during the present study may be applicable to other practical populations and fruit traits observed at OPARC-Nago. Because the block and year effects were not significant and/or negligible,

the environmental variance estimate within a year and a block (σ_E^2) may be applicable to those populations within another year. Breeders normally evaluate fruit traits for cultivars/selections that are commercially important or have potential as cross-parents every year to monitor the year effect. If necessary, breeders can adjust the year effect using data of those cultivars/selections, similar to woody fruit crops (Hansche and Brooks 1965; Yamada et al. 1994a, 1994b, 1995). Assuming a normal distribution of offspring phenotypic values, breeders can easily calculate the percentage of theoretically selected offspring individuals (P_2), which was the nearly the same as the percentage of practically selected offspring individuals (P_1) in the present study.

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