



Short communication

Relationship between inbreeding coefficients and plant height of 1-year-old seedlings in crosses among Japanese pear (*Pyrus pyrifolia* Nakai) cultivars/selections

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ABSTRACT

Plant height, a vigor trait, in 1-year-old seedlings made from Japanese pear (*Pyrus pyrifolia*) cultivars/selections was measured using 994 individuals from 29 families. The family mean of plant height was negatively correlated ($r = -0.72^{**}$) to the inbreeding coefficients (F). The regression of the family mean (F_m) on the F value ($F_m = 130 - 104F$) showed that inbreeding depressions were 8%, 20%, and 40% for $F = 0.1, 0.25,$ and $0.5,$ respectively. According to the regression, the family mean at $F = 0$ was estimated at 130 cm. These results showed that the vigor was greatly influenced by inbreeding in Japanese pear. Within-family variances, the genetic segregation of offspring in a family, differed according to family. The proportions of offspring with plant height above 130 cm (estimated F_m for $F = 0$) were extremely low, i.e., 0–17% for $0.5 \leq F < 0.60$ and 0–8% for $F = 0.75$.

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1. Introduction

Japanese pear (*Pyrus pyrifolia* Nakai) is a major fruit crop in Japan. Genetic improvement of Japanese pear at the Horticultural Research Station (currently, the National Institute of Fruit Tree Science (NIFTS)) started in 1935 (Abe and Kotobuki, 2004). The NIFTS breeding program focused on the improvement of fruit quality (Kajiura and Sato, 1991). Since local cultivars with high quality were restricted to only a few cultivars, crosses over generations had been exclusively made among restricted cultivars/selections (Kajiura and Sato, 1991; Machida and Kozaki, 1975, 1976). Restricted cross-parents and severe selection over generations, however, have resulted in an inbred Japanese pear breeding population.

Inbreeding in cross-pollinated plants causes an increase in the frequencies of homozygosity within an inbred individual. The reduction of characters connected with physiological efficiency due to the expression of deleterious recessive genes results in inbreeding depression. In fruit and berry crops, inbreeding reduces the vigor traits (Bell et al., 1981; Brennan, 1996; Brown, 1975; Brown et al., 1996; Hancock et al., 1996; Iikubo, 1954; Janick et al., 1996; Karnatz, 1988; Leal and Coppens d'Eeckenbrugge, 1996; Lyrene, 1983; Melville et al., 1980; Morrow and Darrow, 1952;

Wilson, 1970). To evaluate the vigor of the plants, plant height has often been measured as an indicator of vigor in fruit and nut crops (Brown, 1975; Lyrene, 1983; Shepard et al., 1989) and forest trees (Bingham and Squillace, 1955; Cram, 1984; Duel et al., 1996; Fowler and Park, 1983; Park and Fowler, 1984; Rudolph, 1981; Snyder, 1968, 1972; Sorensen and Miles, 1982).

Vigor is important for fruit crops because it is crucial to achieving higher yields (Way et al., 1983). In Japanese pear, a loss of vigor leads to less productivity, and recently released cultivars and advanced selections at NIFTS appear to have less yield than local cultivars such as 'Nijisseiki' and 'Chojuro' (Kajiura, 1996; Kotobuki et al., 1991; Kotobuki, 1996; Kotobuki et al., 2004). However, there is no quantitative study on the relationship between inbreeding and vigor in Japanese pear.

In Japanese pear, vigor has been evaluated not by the measurement of plant height but by visual assessment because Japanese pear is trained on a horizontal trellis (Kajiura, 1994). However, evaluation based on visual assessment has the potential to produce misleading results, particularly when the observations are done without yearly repetitions and tree replications. Furthermore, tree vigor in Japanese pear changes by tree age and cultural practices, such as pruning, fertilization, fruit thinning, and irrigation. Therefore, it is not easy to evaluate vigor in adult trees of Japanese pear by visual assessment.

Several reports on forest trees have shown that reduction of plant height due to inbreeding is observed even in 1- or 2-year-old seedlings (Bingham and Squillace, 1955; Cram, 1984; Fowler and

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Park, 1983; Hatakeyama and Ishikura, 1982; Rudolph, 1981; Snyder, 1972; Saito et al., 1986; Sorensen and Miles, 1982). In the Japanese pear breeding system at NIFTS, 1-year-old seedlings are grown in a nursery and not trained on a horizontal trellis, so that the plant height of the seedlings can be easily measured. As for plant height, 1-year-old seedlings are probably less influenced by cultural practices than adult trees because they are not pruned and are in a vegetative phase. If the difference in vigor in Japanese pear could be observed in 1-year-old seedlings, the plant height of 1-year-old seedlings would be available for early screening of tree vigor in selection. The objective of this study was, therefore, to evaluate whether the difference of plant height due to inbreeding could be observed in 1-year-old seedlings in Japanese pear.

2. Materials and methods

The experiment was carried out at the National Institute of Fruit Tree Science (Tsukuba, Ibaraki, Japan). Using 25 cultivars/selections (Table 1), 26 crosses were made in 2003–2005 for the purposes of genetic improvement and of obtaining homozygous individuals for self-incompatibility genes (Table 2). After harvest, seeds were taken from fruits and kept in a refrigerator at 5 °C. The following spring (March), seeds were sown in Jiffy Pots Strips (square, 8 cm × 8 cm, Jiffy) with a soil mixture of 1 sand: 1 bark- and chicken manure-based compost (by volume) in a glasshouse. Plants were grown under natural light and temperature. As of April, side-windows of the glasshouse were opened to avoid rising temperatures. After germination, liquid fertilizer (HYPONEX, HYPONEX Japan) was applied three times with a 30–40 mL solution per pot (1 mL L water, 5N–10P–5K). Neither pesticides nor fungicides were applied while seedlings were growing in the glasshouse. In early to mid-May, seedlings were transplanted to a

Table 1
Japanese Pear (*Pyrus pyrifolia*) genotypes (cultivars/selections) and their parentage used as parents in this study to quantify inbreeding depression effects on plant height

Cultivar/selection	Parents	
	Female	Male
Akemizu ^a	Shinsui	Hosui ^a
Akiakari	162-29	Hiratsuka 17gou
Akizuki	162-29	Kosui
Chikusui	Hosui ^a	Hakkou
Gold Nijisseiki	Bud mutant of 'Nijisseiki'	
Kosui	Kikusui	Wasekouzou
Nikkori	Niitaka	Hosui
Osa Nijisseiki	Bud mutant of 'Nijisseiki'	
Shinsei	Suisei	Shinkou
Shinsetsu	Imamuraaki	Okusankichi
Shuugyoku	Kikusui	Kosui
Taihaku	Unknown	
Tsukuba 51gou	Hosui ^a	269-21
Yasato	Hakko	75-23
162-29	Niitaka	Hosui
269-21	Hosui	Osa Nijisseiki
391-1	Gold Nijisseiki	Nijisseiki ^b
393-1	Chojuro	Chojuro ^b
409-3	Gold Nijisseiki	Nijisseiki ^b
413-3	Shinsui	Kosui
415-1	Kosui	Kosui ^b
416-5	Gold Nijisseiki	Nijisseiki
426-59	162-29	269-21
430-44, 430-91	Hosui	269-21

^a Parentages were according to Sawamura et al. (2004, 2008).

^b Planted in the gamma field of the Institute of Radiation Breeding, National Institute of Agro-biological Science, at various distance from ⁶⁰Co source. In this study, these parental individuals were regarded as same genotype as the original cultivars.

Table 2

Cross-combinations, number of individuals, and inbreeding coefficients in each family to quantify inbreeding depression effects on plant height

Cross-combinations	No. of seedlings	Inbreeding coefficients (<i>F</i>)
Akiakari × Taihaku	49	0.047
Akizuki × 426-59	50	0.217
Chikusui × Taihaku	38	0.063
Chikusui × 426-59	40	0.225
Chikusui × 430-44	22	0.316
Gold Nijisseiki × 391-1	42	0.500
Gold Nijisseiki × 409-3	46	0.500
Gold Nijisseiki × 416-5	50	0.500
Kosui × 430-44	17	0.227
Nikkori × Tsukuba 51gou	35	0.223
Nikkori × 426-59	50	0.219
Osa Nijisseiki × Taihaku	50	0
Shinsei selfing	18	0.563
Shinsetsu × Tsukuba 51gou	37	0
Shuugyoku × 413-3	52	0.282
Shuugyoku × 415-1	58	0.375
Tsukuba 51gou × Chikusui	40	0.316
Tsukuba 51gou × 162-29	50	0.223
Yasato × Tsukuba 51gou	20	0.229
Yasato × 430-44	37	0.193
Yasato × 430-91	21	0.193
269-21 × Akemizu	29	0.227
426-59 × Shinsei	50	0.117
391-1 selfing	25	0.750
393-1 selfing	24	0.750
415-1 selfing	44	0.750

nursery at a spacing of 0.15 m × 1 m. The nursery soil was a humic andosol. Before transplanting, bark- and chicken manure-based compost and fertilizer were applied to the nursery at 7 t ha⁻¹ and at a rate of 50N–50P–50K kg ha⁻¹, respectively. The plant height of 1-year-old seedlings (from the soil level to the tip of the meristem) was measured in December when shoot elongation had stopped. When the stems of plants were bending, their lengths were measured as plant height.

The pedigrees of cultivars/selections used in this study were gathered from various sources such as the documents for Japanese pear cultivars and the records for cross-combinations at the NIFTS, and from several other documents. The parentage of 'Hosui' was according to Sawamura et al. (2004). 'Gold Nijisseiki' and 'Osa Nijisseiki', the bud mutants of 'Nijisseiki', were regarded as the same genotype as 'Nijisseiki'. The pollen parents of 391-1, 393-1, 409-3, and 416-5 were 'Nijisseiki', 'Chojuro', and 'Kosui', planted in the gamma-field of the Institute of Radiation Breeding, National Institute of Agrobiological Sciences, at various distances from the ⁶⁰Co source, and irradiated chronically; however, no differences between the irradiated trees and the original cultivars were found by visual observation. In this study, these irradiated pollen parents were regarded as the same genotype as the originating cultivars. The parents of 'Akemizu' were released as 'Shinsui' and 'Hiratsuka 17gou', but S-genes and SSR analyses revealed that the parents were 'Shinsui' and 'Hosui' (Sawamura et al., 2008). Therefore, the parents of 'Akemizu' were determined to be 'Shinsui' and 'Hosui'.

The inbreeding coefficient of an individual (*X*) were calculated according to Falconer (1989) using the formula:

$$F_X = \sum (1/2)^n (1 + F_A),$$

where *n* is the number of individuals in any path of relationship counting the parents of *X* and the common ancestor. *F_A* is the inbreeding coefficient of the common ancestor.

The linear regression equation was calculated using the family mean (*F_m*) as a dependent variable and the *F* as an independent variable. Inbreeding depression was estimated as follows:

Table 3Proportions of offspring with plant height more than 130 cm^a in 1-year-old seedlings in 26 families with various inbreeding coefficients (*F*) in Japanese pear

Cross-combination	Proportion in each family (%)	Cross-combination	Proportion in each family (%)
<i>F</i> = 0			
Osa Nijisseiki × Taihaku	62	Shinsetsu × Tsukuba 51gou	57
0 < <i>F</i> < 0.1			
Akiakari × Taihaku	31	Chikusui × Taihaku	29
0.1 ≤ <i>F</i> < 0.2			
Yasato × 430-44	30	Yasato × 430-91	29
426-59 × Shinsei	26		
0.2 ≤ <i>F</i> < 0.3			
Akizuki × 426-59	30	Chikusui × 426-59	0
Kosui × 430-44	71	Nikkori × Tsukuba 51gou	89
Nikkori × 426-59	30	Shuugyoku × 413-3	58
Tsukuba 51gou × 162-29	20	Yasato × Tsukuba 51gou	5
269-21 × Akemizu	28		
0.3 ≤ <i>F</i> < 0.4			
Chikusui × 430-44	68	Shuugyoku × 415-1	3
Tsukuba 51gou × Chikusui	10		
0.5 ≤ <i>F</i> < 0.6			
Gold Nijisseiki × 391-1	0	Gold Nijisseiki × 409-3	17
Gold Nijisseiki × 416-5	0	Shinsei selfing	6
<i>F</i> = 0.75			
391-1 selfing	0	393-1 selfing	8
415-1 selfing	0		

^a 130 cm is family mean for *F* = 0 estimated from the regression, $F_m = 130 - 104F$, as shown in Fig. 1. *F_m*: family mean, *F*: inbreeding coefficient.

$(F_m0 - F_m i) \times 100/F_m0$, where *F_m0* and *F_mi* are the estimated family means for *F* = 0 and *i*, respectively. To confirm the linearity of plant height to *F*, a curvilinear regression (quadratic) was also calculated. The homogeneity of within-family variances (variance due to segregation in a family) was tested by Bartlett's test (Snedecar and Cochran, 1972). A total of 994 individuals over families were used for the study.

3. Results

The *F* values in each family ranged from 0 to 0.75 (Table 2), and the mean *F* value over families was 0.308. The family mean for plant height varied depending on the cross-combination, ranging from 32 cm (391-1 selfing) to 150 cm ('Nikkori' × Tsukuba 51gou). The linear regression of the family mean (*F_m*) on the *F* value was highly significant, but the curvilinear regression was not significant. As a result of the linear regression analysis, the equation, $F_m = 130 - 104F$, was obtained (Fig. 1). According to the regression, the estimated *F_m0* was 130 cm, and the inbreeding depressions were estimated to be 8%, 20%, and 40% for *F* = 0.1, 0.25, and 0.5, respectively.

Within-family variances ranged from 166 (391-1 self) to 1989 (269-21 × 'Akemizu'). Bartlett's test showed that the within-family variances differed significantly among families. Within-family variances were correlated neither with the family means ($r = 0.10^{NS}$) nor the *F* levels ($r = -0.25^{NS}$). Thus, no trend was found in the relationship between the within-family variance and the family means or *F* levels.

The proportions of offspring with plant height above *F_m0* (130 cm) varied even at similar *F* levels but generally decreased as the *F* levels increased (Table 3). Particularly, the proportions were extremely low, i.e., 0–17% for $0.5 \leq F < 0.60$ and 0–8% for *F* = 0.75.

4. Discussion

The plant height of seedlings in Japanese pear was greatly reduced by inbreeding, even in 1-year-old seedlings, and was

inversely proportional to the *F* level. This trait is an effective indicator in an early screening of tree vigor in Japanese pear breeding.

In several fruit crops, a loss of vigor as a result of inbreeding is useful for vigor controls, including dwarfing (Brown et al., 1996; Karnatz, 1988), while greater vigor is crucial to achieving higher yields and is important for propagation, establishment, rapid fruit growth, and recovery from stress (Way et al., 1983). In Japanese pear, the varietal difference of tree vigor did not correspond to that in internode lengths, a tree habit; for example, Nijisseiki' has short internodes but is vigorous (Ibaraki Prefecture, 1978). Moreover, we also observed that highly inbred Japanese pear seedlings bore only a few fruits due to reduced vigor even when the tree was aged and that some plants had died (unpublished data). This suggested that controlling tree habit by inbreeding is difficult for Japanese pear.

Inbreeding depression for *F* = 0.25 and 0.5 was estimated to be 20% and 40%, larger than the values previously reported for the plant height of 1-year-old seedlings in forest trees (Bingham and Squillace, 1955; Fowler and Park, 1983; Rudolph, 1981; Snyder, 1968, 1972). However, the reason for this large depression is unknown. According to Falconer (1989), the main reason for a change in the mean value caused by inbreeding depression is directional dominance, which means that the dominance of the genes concerned is preponderantly in one direction. Moreover, when loci combine additively, the change in the mean on inbreeding should be directly proportional to the inbreeding coefficient (Falconer, 1989). As shown in Fig. 1, the result showed that the relationship between the *F* and plant height was linear, suggesting that the genes related to vigor in the Japanese pear act additively.

In fruit breeding, once a superior offspring is selected, the individual is vegetatively propagated. If the within-family variance is large, the proportion of obtaining a superior offspring is not small even when the family mean does not exceed a given selection criterion. Thus, the proportion of obtaining a superior offspring in fruit breeding depends not only on the family mean but also on the

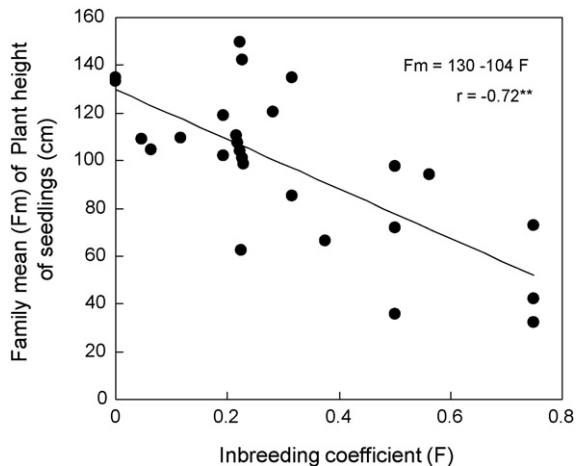


Fig. 1. Relationship between inbreeding coefficients and family mean of plant height of 1-year-old seedlings derived from 26 families in Japanese pear cultivars/selections. **Significant at $P < 0.01$.

within-family variance. In this study, within-family variances were varied depending on the families. In general, there may be two primary reasons for the heterogeneity of the within-family variance. First, when the variances are proportional to the mean values, the dependence can disappear after log-transformation. In such cases, the dependence of the variance on the mean is called a 'scale effect' (Falconer, 1989). In this study, the within-family variance was not correlated to the family mean, indicating that the heterogeneity of the variances was not due to the scale effect. Second, within-family variances theoretically should decrease as a result of inbreeding, but a significant relationship was not found between the F level and the variance. Thus, the reason for the heterogeneity of the variances was not obvious. To clarify the cause of the heterogeneity of the variance, a careful mating design and a large number of offspring will be needed.

The proportion of offspring with plant height above 130 cm was low in highly inbred crosses, particularly in a cross in which the F value exceeded 0.5. This indicates that the probability of offspring with plant height similar to $F = 0$ is low due to selfing.

In conclusion, the variation in the family mean of plant height in 1-year-old seedlings in Japanese pear could be largely explained by the degrees of inbreeding, although the segregation in a family varied among families, even within the same F level. Therefore, when conducting crosses among related individuals, the breeder should pay attention to plant height in a nursery to prevent new cultivars/selections from losing vigor.

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