# Response to Inbreeding of Early Seedling Growth and Fruit Traits in a *Prunus domestica* L. Breeding Population

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## Abstract

**This is a continuation of an inbreeding study done in 2002 that tested for the presence of inbreeding depression in a *Prunus domestica* (European plum) breeding population at the University of California at Davis. That study found inbreeding depression affecting seed germination and growth traits in the first season of growth. This study tested the same population for inbreeding depression in relation to field survival (2003-2008), young tree growth traits (height (2004) and cross sectional trunk area (2004-2008)), and fruit traits (fruit set (2004-2008), fruit size and soluble solids content at harvest (2007)). The test population was created with controlled crosses using 20 elite parents. The final population was made up of 11 self-pollinated families (inbreeding coefficient F=0.5), 10 full-sibling families (F=0.25), 11 non-related crosses (F=0) and a control set of random-matings (average F=0.23) that had accumulated inbreeding over 2 to 5 generations with intervening cycles of selection. In spring of 2003, part of each treatment group was planted at either 0.79 m spacing or 1.22 m spacing within a randomized block design at the UC Davis pomology orchards. Survival of seedlings from self-pollinated parents was significantly lower (89%) than non-related progenies (97%). Tree spacing significantly affected tree growth and time of first fruit set, with the 0.79 m spaced trees being smaller but setting fruit a year earlier than trees at the 1.22 m spacing. Trees resulting from greater inbreeding had smaller mean trunk cross sectional areas when grown at the 1.22 m spacing.**

## INTRODUCTION

Successful breeding of fruit trees requires substantial inputs of time, labor and economic resources. Genetic factors of the breeding population that reduce the effectiveness of breeding decisions or lengthen the selection process can compromise the ultimate success of the cultivar development process. Most breeding programs have relatively few elite selections and cultivars from which to select parents for subsequent generations. Finite parental population size increases the likelihood of relatedness within the selected parents and inbreeding for their progeny. A negative consequence of inbreeding may be inbreeding depression for traits of commercial importance. Inbreeding depression is defined as the reduction in the mean of a metric trait in a population and is associated with lower vigor, fertility, and survival. The change in the population mean is caused by the accumulation of homozygosity due to identity by decent of alleles that have been inherited through a common ancestor (Falconer and Mackay, 1996). If inbreeding depression exists for important traits, and a substantial fraction of the breeding population carries excess homozygosity due to inbreeding, the selection process may take longer and proceed with reduced chance of success. The amount of inbreeding accumulated in the progeny of related parents is quantified by the inbreeding coefficient, F, which includes the consequences of inbreeding in the current generation and inbreeding accumulated over past generations (Falconer and Mackay, 1996). Knowledge of the effects expected due to inbreeding for any specific breeding population is required for breeders to maximize selection response relative to program effort.

This study is a continuation of an inbreeding study performed within the *Prunus* *domestica* breeding population at UC Davis completed in 2002 (DeBuse et al., 2005). Briefly, the 2002 study examined the effect of inbreeding on germination and early growth traits by comparing inbred progenies to nonrelated progenies. Comparisons were also made with a randomly mated control set of progenies that had accumulated inbreeding over multiple generations with intervening cycles of selection. This study detected inbreeding depression in current-generation inbred progenies for germination rate as well as for all the growth traits measured. Significant regression of trait means on F was not detected for the control set, where inbreeding had accumulated over generations, suggesting that intervening cycles of selection can have a powerful effect on mitigating consequences of accumulated homozygosity for trait means.

Although our preliminary results demonstrated inbreeding depression for germination and first year growth traits, there are many questions as to how inbreeding may influence the growth traits and survival as the progenies mature. In a long term study (29 years) of Ponderosa pine (*Pinus ponderosa* L.), the mean of the tree height for individual families was similar over environments but the means among families differed at different stages of life. The authors concluded that genetic expression of height growth changes over time and family performance was modified by inter-tree competition (Namkoong and Conkle, 1975). Though inbreeding was not examined in this study, their findings showed that at different stages of life different genes may be expressed.

The most important traits for fruit tree breeding are yield and quality of the fruit. Inbreeding depression for fertility may affect precocity, the tendency of trees to set fruit in the early years, and may reduce crop size at maturity. The 2002 study indicated that progenies with inbreeding accumulated gradually did not exhibit the same reduction in the measured traits as the current-generation inbred progenies. The current study evaluated trait mean depression due to inbreeding as maturity approached, using the same trees and monitoring them for 6 years in an experimental orchard at UC Davis.

A secondary objective of this study is to determine whether inbreeding depression is exhibited differently in different growing conditions. An inbreeding study of Scots pine (*Pinus sylvestris* L.) grown in two environments, found differences between the same families for survival and tree height but the resulting trends of inbreeding depression were the same over both environments (Koelewijn et al., 1997). In this *P. domestica* study, two similar plots were planted with the only difference being the distances between the trees (tree spacing). This was designed to test if tree-to-tree competition affected growth and fruit traits and/or magnitude of inbreeding depression of these same traits.

## MATERIALS AND METHODS

The same trees used for the 2002 study were used in this study. Four sets of progenies with different levels of inbreeding were generated by controlled pollinations made in 2001 using genetic materials from the UC Davis *P. domestica* breeding program. Twenty parent trees were chosen that had at least one full sibling within the group. This parental group originated from nine parental trees which in turn originated from sixteen grand-parental trees. From this parental group, three levels of current-generation inbreeding were created: 11 self-pollinated (selfed), 10 full-sibling (full-sib), and 11 nonrelated progenies. A fourth progeny group was created to reflect random mating within the parental group. These progeny were the control set that had accumulated inbreeding over multiple generations with intervening cycles of selection. The amount of relatedness for current-generation inbred progenies was quantified by the inbreeding coefficient, F, with self-pollinated progenies having F = 0.50, full-sib F = 0.25, and crosses among unrelated parents F = 0.00 (Falconer and Mackay, 1996). Because of the small breeding population and the past inbreeding, the nonrelated progenies actually had F = 0.0909, but their parents were not related in the prior generation (F was calculated using SAS procedure Inbreed (SAS, 1999)). The control set of progenies had F values ranging from 0.1875 to 0.3438 and a group mean F =0.2299.

The detailed protocol of pollination, germination, and nursery planting is reported in the materials and methods for the 2002 study (DeBuse, 2005). This current study began at the planting of the bare-rooted trees after a short time in winter storage in an outside sawdust box. The trees were field planted in February of 2003 at the UC Davis Pomology Orchards, Davis, California (lat. 38°32' N, long. 121°47'W; elev. 20.4 m). The location had been previously used as a peach orchard and was not fumigated between plantings, but was left fallow for two years prior to planting. Soil type is Rincon silty clay loam (USDA and NRCS, soil survey).

The experiment was planted in two adjacent plots; within-row tree spacings of 0.79 and 1.22 m were used for these plots. Rows were 4.57 m apart. Each plot was established using a randomized complete block design with four blocks. Each block contained equal numbers of the progenies from each family within inbreeding treatments. The same cultural practices were applied for both plots. Irrigation was provided by micro-sprinkler. Standard commercial fertilizer and pest management programs were implemented.

Individual tree survival was recorded in September 2008. In 2004, tree height and trunk diameter, 2 inches above the soil, were measured. In 2006 and 2007 trunk diameter, 2 inches above the soil, was again measured. Trunk diameter was used to calculate the trunk cross sectional area (TCA), which was used for further analyses. Fruit set and crop size data were collected by rating each tree on a subjective scale of 1-9 for the amount of fruit on the tree (1 = a few fruit, 5 = average commercial crop, 9 = heavy crop). In the summer of 2007, a sample of 10 fruit per tree was randomly harvested throughout the plots. Average fruit internal flesh pressure was measured to confirm that all samples were within harvest maturity of between 1.7 and 3.1 N/cm$^2$. Average fruit weight and soluble solids content were recorded for fruit samples as they met this maturity requirement.

Differences among the survival rates for the inbreeding treatments were tested using Chi Square. Progeny means of the measured growth traits for inbreeding treatments were analyzed using a fixed effect analysis of variance (ANOVA) with SAS Procedures GLM (SAS, 1999) (Tables 1 and 2). Duncan's multiple range test was used to establish levels of significance between individual treatments. The variances of progenies nested within inbreeding treatment were examined using the random effects ANOVA (data not shown).

## RESULTS AND DISCUSSION

Significant differences for survival percentage were found among inbreeding treatments at all growth stages (germination, nursery, and field) (Table 3). Germination results reported in the 2002 experiment demonstrated significant differences ($\chi^2$= 87.9, P<0.01) for all inbred progenies compared to nonrelated progenies. The non-inbred progenies had 82.3% survival while the full-sibs had 69.9%, selfed progenies 60.9% and the control set 67.3%. A small but significant difference in survival was found between treatments in the nursery ($\chi^2$= 5.12, P=0.016). There were no significant differences between nonrelated progenies (97.3%), full-sib (95.2%) and selfed (95.3%) progenies, respectively. The control set, with 93.9%, was only treatment with significantly smaller survival percentage than the nonrelated progenies, but it did not differ significantly from the other two treatments. The lower mortality in the nursery may have been due to the careful management of nutrients, water and weeds in the nursery, that probably reduced survival stresses. Similar findings were reported by Libby et al. (1981) while studying the consequences of inbreeding in redwood, where survival rates for selfed progenies were higher in the nursery than for the field. The final survival for our study was recorded in 2008 after the seedlings had been in the field for 6 years. There was no difference in survival percentage related to tree spacing. However, significant differences were found among the survival rates of the four inbreeding treatments ($\chi^2$= 24.3, P<0.01) with the survival rate for nonrelated progenies, 97.4%, being significantly higher than the other three treatments. Survival rates for full-sib (93.3%) and the control set (92.6%) did not differ significantly from each other. The lowest percent survival in the field was in the selfed progenies (88.6%), which was significantly lower than all other treatments.

Survival results show that mortality increases with inbreeding coefficient for this population of *P. domestica*, for both current-generation inbreeding and for inbreeding accumulated over generations. The reduction of viability at germination may be caused by increased homozygosity for lethal recessive alleles or by increased expression of unfavorable alleles that reduce the individual's vigor. In the later stages of growth, reduced stability is most likely due to the increase in homozygosity for unfavorable recessive alleles related to vigor. The causes of mortality in this study were not determined, but in a similar inbreeding study of black spruce (*Picea mariana* (Mill.)) the authors, Park and Fowler (1983), presumed that embryonic lethal recessives caused the mortality of inbred progenies and estimated the numbers of lethal recessives to range from 0.5 to 14.1 per zygote. Whatever the cause for the decreased survival, it is likely that the more homozygous individuals are ultimately being removed from the population. The selective removal of these individuals is expected to reduce the magnitude of the inbreeding depression exhibited in the metric traits of breeding population (Sorensen and Miles, 1982; Koelewijn et al., 1999).

Significant differences were found among the inbreeding treatments for all growth traits measured (height 2004, TCA 2004, 2006, 2007) and between the 0.79 and 1.22 m tree spacings in all years (Tables 1 and 2). Means for the growth traits and the percentage decrease compared to the nonrelated progenies are found in Tables 4-6. The trees planted at 0.79 m were significantly smaller than the trees planted at 1.22 m ($p \leq 0.01$) for all growth traits. Closer spacing resulted in a 10% decrease in height when averaged over all the treatments and 33% decrease for TCA in 2004, with a 20% difference in 2006 and 2007. Inter-tree competition for resources explains the decreased in size and vigor of the trees and this competition increased the magnitude of inbreeding depression found in the selfed progenies. For example, the heights of selfed progenies were 16.6% less than the nonrelated progenies and TCAs were 22-29% less at the 0.79 m spacing. Height for the selfed progenies at 1.22 m was reduced by 7.9% and TCAs were 19-22% less than for nonrelated progenies. For full-sib progenies the height was 13.9% less than non-related progenies at the 0.79 m spacing and 11.8% at the 1.22 m spacing; the TCAs seemed to show an inverse relationship with spacing, 13-16% smaller TCA at 0.79 m spacing and 16-21% smaller at 1.22 m. The reason for the increased inbreeding depression in the wider spaced plot for the TCAs of the full-sibs is not clear, but the overall results show that inter-tree competition can affect the magnitude of the expression of inbreeding depression.

Significant differences were found among inbred treatments within each spacing treatment. Selfed and full-sib progenies were significantly smaller than nonrelated progenies for height and TCAs for all years. Differences for height and TCA in 2006 at the 1.22 m spacing and TCAs in 2004 and 2006 were not statistically significant. The selfed progenies were significantly smaller than the full-sib for TCA in 2004 at the 0.79 m spacing and in 2007 for both spacings. These results are consistent with the findings of the 2002 trial, where inbreeding depression was found for all vegetative growth traits ranging from 2-9% (2 month) and 6-20% (10 month) for full-sibs and 14-30% (2 month) and 13-28% (10 month) for selfed progenies.

No significant difference was found between nonrelated progenies and the control set progenies for any of the growth traits. These finding are similar to those obtained from the regression analysis of these progenies in the 2002 study, where no relationship was found between the inbreeding coefficients (F) when compared to the mean seedling growth traits. Selection at each breeding cycle appears to mitigate the cumulative negative effects of inbreeding in this *P. domestica* population. This relationship was similar to that detected in a strawberry (*Fragaria ananassa*) breeding population (Shaw, 1995).

The 2002 study showed that variances for growth traits of the progenies nested in the inbreeding treatment were greater than variances among the inbreeding treatments themselves. The current study compared variances for the growth traits of more mature trees using random effects ANOVA. This analysis revealed similar patterns (data not shown). Large variances among progenies within the inbreeding treatments may indicate that there may be different responses to inbreeding within the breeding population (Shaw, 1997), or may simply reflect the substantial genetic variation for growth traits that were expressed in this population regardless of inbreeding status.

Initial fruit set or precocity of the seedlings was scored as the percentage of trees with fruit within each inbreeding treatment; the analysis was separated by tree spacing (Fig. 1). Trees for all inbreeding treatments were more precocious at the 0.79 m spacing, which began bearing in 2004, than for trees at 1.22 m, that began bearing in 2005. By 2007, there were no differences between the two tree spacings for the number of trees that bore fruit. These difference in the first years were more likely due to the reduced vigor and earlier maturity in the closely planted trees caused by inter-tree competition than the inbreeding treatments.

Significant differences were found among inbreeding treatments for the average crop size in the years 2006, 2007 and 2008 ($p \leq 0.01$ for each year). There were no significant differences found in 2004 or 2005 for crop size among inbreeding treatments. Also, no significant differences were found between the two tree spacings for crop size for any year (Fig. 2). There were no significant differences in crop size between nonrelated and control set progenies for any year. This again indicates that accumulated inbreeding over multiple generations with intervening cycles of selection mitigated the magnitude of inbreeding depression observed not only for growth traits but for fruit yield as well. Crop sizes for full-sib and selfed progenies were significantly less than for the nonrelated progenies but were not significantly different from each other in any year. Thus crop size was affected by inbreeding depression for current-generation inbred progenies. This result is similar to the reduced flowering and yields that were found due to current-generation inbreeding in strawberries (Shaw, 1995, 1997), while the reduction was mitigated if selection was done in the parental generations (Shaw, 1997).

In 2007, average fruit size and average soluble solids content were obtained for 201 seedlings distributed randomly over all experimental blocks. The ANOVA results indicated no significant differences among inbreeding treatments for either of these traits (data not shown). Because the fruit was collected over the season as the fruit matured and because the fruit flesh firmness varied over a range of 1.7 and 3.1 $N/cm^2$, these data could not be used to indicate the level of inbreeding depression for fruit quality traits. These traits should be carefully examined with a smaller range of maturity indices over multiple years to give a clear conclusion regarding fruit quality traits.

## CONCLUSIONS

This study demonstrated inbreeding depression for growth traits and fruit yield in *P. domestica* due to current-generation inbreeding. The study indicates that the magnitude of inbreeding depression can be managed by controlling the severity of inbreeding at each mating cycle, and the cumulative consequences of inbreeding can be mitigated by selection for vegetative plant vigor at each cycle. Germination and survival rates were also affected by inbreeding and trait mean depression for these characters occurred at each cycle where mating among relatives took place. Breeders should be aware that as the relatedness of the population increase due to finite population size that mortality rates for seed and young trees could increase. Insuring cross pollination by doing hand pollinations is an expensive and difficult step in breeding trees. However the number of seed produced by this method is crucial to the breeding process because it can reduce inbreeding that occurs randomly in open pollinated seedlings. Inbreeding should be minimized so that a larger number of viable seedling trees can be produced, thus increasing potential for success in future selection cycles.

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## Tables

Table 1. Analysis of variances for *P. domestica* inbred progenies for tree height measured, 2004, planted at two tree spacing, 0.79 and 1.22 m.

| Source | df | Height (both spacing) | |
|---|---|---|---|
| | | Mean square | F |
| Block | 3 | 34439 | 14.42** |
| Tree spacing | 1 | 88775 | 37.16** |
| Inbreeding treatment | 3 | 51508 | 21.56** |
| Inbreeding treatment × tree spacing | 3 | 3368 | 1.41 |
| Error | 1216 | | |

*,** P at 0.05 and 0.01 levels of significance respectively.

Table 2. Analysis of variances for *P. domestica* inbred progenies for trunk cross sectional area (TCA); 2004, 2006, and 2007; planted at two tree spacing, 0.79 and 1.22 m.

| Source | df | TCA 2004 | | TCA 2006 | | TCA 2007 | |
|---|---|---|---|---|---|---|---|
| | | Mean square | F | Mean square | F | Mean square | F |
| Block | 3 | 582948 | 3.33* | 2485023 | 2.28 | 1837405 | 0.70 |
| Tree spacing | 1 | 21834039 | 124.64** | 52173054 | 47.81** | 116619219 | 44.42** |
| Inbreeding treatment | 3 | 2642927 | 15.09** | 19664910 | 18.02** | 40966833 | 15.60** |
| Inbreeding treatment × tree spacing | 3 | 253559 | 1.45 | 640691 | 0.59 | 1672187 | 0.64 |
| Error | 1217 | | | | | | |

*,** P at 0.05 and 0.01 levels of significance respectively.

Table 3. Percentages of survival of the *P. domestica* inbred progenies and control set of randomly mated progenies relative to the nonrelated progenies for the germination, first season growth in the nursery and over first 6 years in the field.

| Inbreeding treatment | Germination | | Nursery 2002 | | Field 2008 | |
|---|---|---|---|---|---|---|
| | % Geminated seed | % Germinated relative to nonrelated progenies | % Survival | % Survival relative to nonrelated progenies | % Survival | % Survival relative to nonrelated progenies |
| Non-related progenies | 82.3 a[1] | | 97.3 a | | 97.4 a | |
| Full-sib progenies | 69.9 b | 85 | 95.2 ab | 97.8 | 93.3 b | 95.7 |
| Selfed progenies | 60.9 c | 74 | 95.3 ab | 97.6 | 88.6 c | 90.9 |
| Control set progenies | 67.3 bc | 82 | 93.9 b | 96.5 | 92.6 bc | 95.4 |

[1] Letters indicate the significant differences of treatment means separated by Chi Square.

Table 4. Means (±SD) of the *P. domestica* inbred progenies and control set of randomly mated progenies at 0.79 and 1.22 m tree spacings and the relative decrease of the means to the nonrelated progenies for the growth traits for tree height (cm) in 2004.

| Inbreeding treatment | Tree spacing (m) | n | Height (cm) 2004 | |
|---|---|---|---|---|
| | | | Mean | % Decrease |
| Nonrelated | 0.79 | 290 | 187.8±44.8 a[1] | |
| Full-sib | 0.79 | 141 | 161.7±43.3 b | 13.9 |
| Selfed | 0.79 | 76 | 156.7±57.1 b | 16.6 |
| Control set | 0.79 | 50 | 186.5±39.8 a | 0.7 |
| Nonrelated | 1.22 | 190 | 204.6±50.7 a | |
| Full-sib | 1.22 | 190 | 180.6±50.3 c | 11.8 |
| Selfed | 1.22 | 172 | 188.5±57.4 bc | 7.9 |
| Control set | 1.22 | 119 | 197.4±52.0 ab | 3.5 |

[1] Letters indicate the significant differences between treatment means at the level p≤0.05 using Duncan's multiple range test.

Table 5. Means (±SD) of the *P. domestica* inbred progenies and control set of randomly mated progenies at 0.79 m. tree spacing and the relative decrease of the means to the nonrelated progenies for the growth traits for trunk cross sectional area (TCA) ($mm^2$) in 2004, 2006, and 2007.

| Inbreeding treatment | n | TCA ($mm^2$) 2004 | | TCA ($mm^2$) 2006 | | TCA ($mm^2$) 2007 | |
|---|---|---|---|---|---|---|---|
| | | Mean | % decrease | Mean | % decrease | Mean | % decrease |
| Nonrelated | 290 | 710±18 a[1] | | 2038±51 a | | 2974±83 a | |
| Full-sib | 141 | 598±26 b | 16 | 1760±73 b | 13 | 2601±114 b | 13 |
| Selfed | 76 | 555±42 c | 22 | 1501±118 b | 26 | 2099±200 c | 29 |
| Control set | 50 | 663±41 ab | 7 | 2155±144 a | +6 | 3090±223 a | +4 |

[1] Letters indicate the significant differences between treatment means at the level p≤0.05 using Duncan's multiple range test.

Table 6. Means (±SD) of the *P. domestica* inbred progenies and control set of randomly mated progenies at 1.22 m. tree spacing and the relative decrease of the means to the nonrelated progenies for the growth traits for trunk cross sectional area (TCA) ($mm^2$) in 2004, 2006, and 2007.

| Inbreeding treatment | n | TCA ($mm^2$) 2004 | | TCA ($mm^2$) 2006 | | TCA ($mm^2$) 2007 | |
|---|---|---|---|---|---|---|---|
| | | Mean | % decrease | Mean | % decrease | Mean | % decrease |
| Nonrelated | 190 | 1060±36 a | | 2561±78 a | | 3707±122 a | |
| Full-sib | 190 | 840±31 b | 21 | 2114±74 b | 17 | 3116±116 b | 16 |
| Selfed | 172 | 829±39 b | 22 | 2059±93 b | 20 | 2997±137 c | 19 |
| Control set | 119 | 1024±49 a | 3 | 2609±122 a | +2 | 3760±180 a | +1 |

[1] Letters indicate the significant differences between treatment means at the level p≤ 0.05 using Duncan's multiple range test.

## Figures

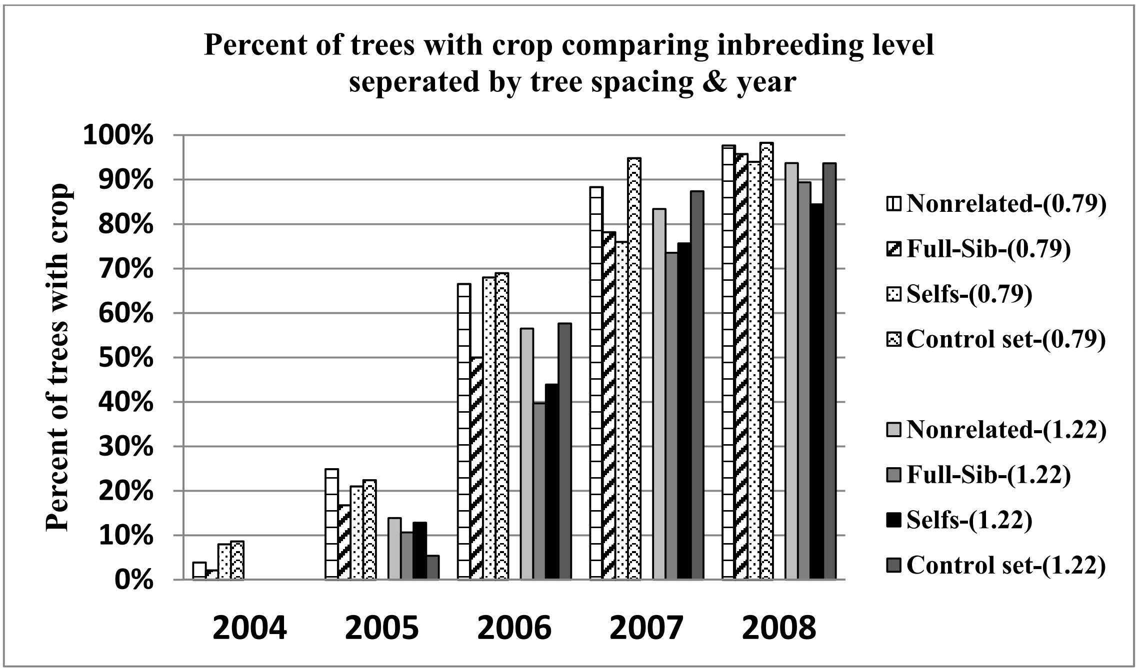

Fig. 1. Percent of trees with crop for the *P. domestica* inbreeding treatment progenies (nonrelated, full-sib, selfed, and control set of randomly mated) separated by year, 2004-2008, and tree planting spacing, 0.79 and 1.22 m.

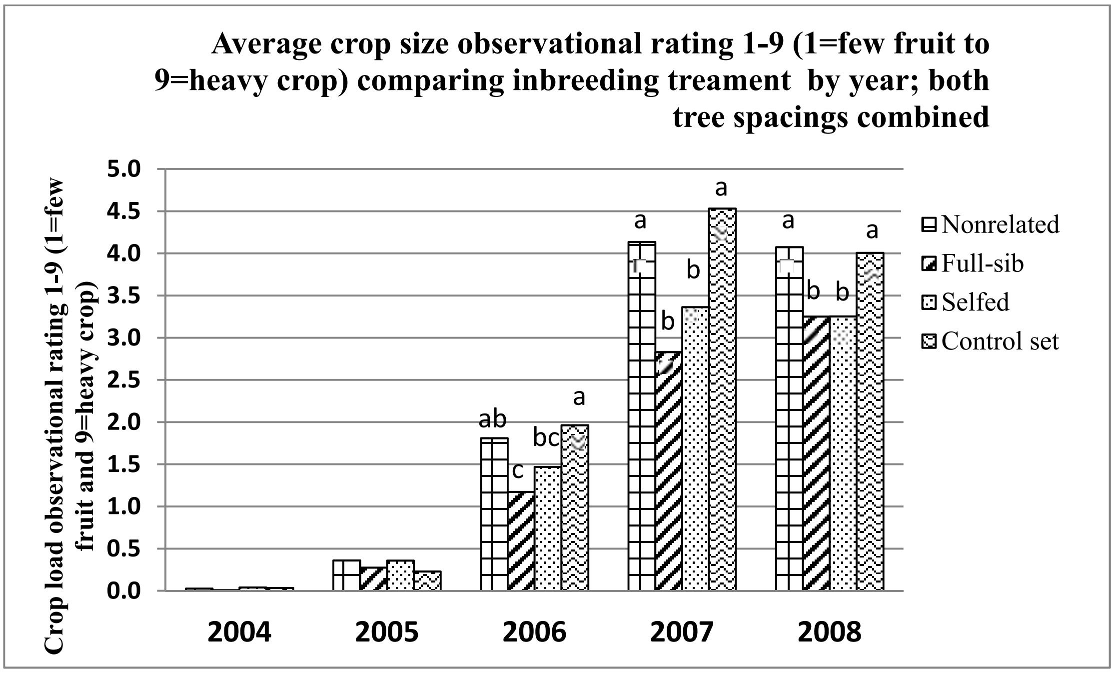

Fig. 2. Average crop load observational rating for the *P. domestica* inbreeding treatment progenies (nonrelated, full-sib, selfed, and control set of randomly mated) separated by year, 2004-2008. Averages combined both tree planting spacing of 0.79 and 1.22 m. Letters indicate the significant differences between treatment means at the level $p \leq 0.05$ using Duncan's multiple range test.