Viability of the use of inbred progenies in recurrent selection in perennial plants

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Abstract: *The viability of using inbred progenies, i.e., S_1 or S_2, in recurrent selection programs for perennial plants was evaluated in comparison with full sibs using the expression of gain from selection. Populations with different genetic properties, were considered in obtaining the estimates, with the population genetic variance always equal to 1 used as reference, as well as different experimental strategies. The estimates of genetic covariances between average additive effects of the alleles and the dominance effects of homozygotes, which occur when S_1 or S_2 is used, although negative for the lower values of the average allele frequencies, were always smaller than the additive genetic variance. Per unit of time, the total gain from selection with S_1 was greater than with S_2 and full sibs. However, it is argued that the best strategy is a cycle with S_1, followed by a cloned progeny test with full sibs.*

Keywords: *Plant breeding, quantitative genetics, comparison of selection methods, genetic variance, average degree of dominance*

INTRODUCTION

Currently, one of the focuses in the breeding of most plant species is increasing the gain from selection per unit of time (Fang et al. 2021, Wanga et al. 2021, Samantara et al. 2022, Sharma et al. 2022). In perennial plants, such as eucalyptus, some alternatives have already been successfully used for some time. Among them are, for example, the process for reducing the time for the plant to begin flowering, and thus the artificial hybridizations can be performed more rapidly (Assis et al. 2005, Castro et al. 2021). The cloned progeny tests have been another alternative because they allow the progeny test and the clonal test to be conducted simultaneously (Resende 2002, Ramalho et al. 2021). There are also studies aiming at routine use of genomics in the selection process (Resende et al. 2017).

However, even without reducing the time of each selection cycle, the gain per unit of time can be increased. In this case, one of the alternatives is using progenies that release a greater proportion of genetic variance. This is the case, for example, in the use of inbred progenies, as occurs in self-pollinating plants and even in annual allogamous plants. In perennial plants, including eucalyptus, noninbred progenies, such as half sib and full sib have been most used in the selection process up to now. In this case, the genetic variance to be exploited from selection is low (Hallauer et al. 2010). In addition, there is evidence that perennial plants must have a high frequency of harmful alleles that are not

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expressed, as they are in heterozygosity. When plants are self-pollinated, although their growth, in volume, decreases (Bison et al. 2006, Costa-Silva et al. 2010), harmful alleles are exposed, allowing them to be eliminated with selection and providing greater genetic variance.

Nevertheless, it should be emphasized that when inbred progenies are used in an intrapopulation recurrent selection (IRS) program, special attention should be given, above all, to one of the components of genetic variance, D_1, which is the genetic covariance between the average additive effects of the alleles and the dominance effects of the homozygotes. As it is a covariance, D_1 can be negative, and as it participates in the numerator of the expression for gain from selection, depending on its magnitude, it may decrease instead of increase the gain from selection expected from IRS (Souza Júnior 2001).

Estimates of D_1 for several traits in annual plants, in addition to the nonexpressive magnitude, were negative (Ramalho et al. 2012, Marques et al. 2022). In eucalyptus, a single report of D_1 estimation showed that the value was also negative (Costa-Silva et al. 2010). For the estimate of D_1, dominance must occur in the expression of the trait, and its magnitude will depend on the allele frequencies in the population (Vencovsky et al. 2001, Resende 2015). Thus, it would be important to assess the consequences of the use of inbred progenies, considering different average allele frequencies of the populations and average degree of dominance in the success expected from IRS using inbred progenies.

A few reports of comparisons of the use of inbred progenies with noninbred progenies in IRS for perennial plants were reported by Resende and Vencovsky (1992). In these comparisons, various factors can have an impact, including the population used (average frequency of the favorable alleles), the type of allele interaction, and the heritability. There are other factors that have an impact, such as the experimental strategy and the number of plants per progeny. The expression of gain from selection used to compare IRS methods has been applied both in self-pollinating plants (Atlin and Econopouly 2022, Marques et al. 2022) and in annual allogamous plants (Hallauer et al. 2010). It should be highlighted that when IRS is performed using inbred progenies, i.e., S_1 or S_2, the selected individuals should be recombined by crossing the plants in pairs, obtaining FS progenies.

As already mentioned, except for Resende and Vencovsky (1992), reports of comparisons among recurrent selection methods using inbred progenies were not found. In light of the above, the aims of this study were to assess the implications of using inbred progenies (S_1 or S_2) to estimate the different components of genetic variance, especially D_1, in this situation, considering different average degrees of dominance and average allele frequency ($\bar{p}$) for the traits under selection in the population and heritability. In addition, estimates of gain from selection will be obtained involving inbred progenies and FS progenies, as well as through use of some experimental strategies.

MATERIAL AND METHODS

Theoretical development

In the comparisons, inbred progenies (S_1 and S_2) were considered in an IRS program applicable to any species. The reference was an S_0 population in Hardy–Weinberg equilibrium of a pure species or obtained from the S_0 generation derived from the crossing of clones (Figure 1).

The genetic variance (σ_G^2) of a population under inbreeding can be decomposed, as presented by Souza Júnior (1989), as $\sigma_G^2 = (1 + F)\sigma_A^2 + (1 - F)\sigma_D^2 + 4FD_1 + FD_2 + F(1 - F)\breve{H}$, where F is the inbreeding coefficient, σ_A^2 is the additive genetic variance, σ_D^2 is the dominance variance, D_1 is the genetic covariance between the average additive effects of the alleles and the dominance effects of the homozygotes (as it is a covariance, it can assume positive or negative values), D_2 is the genetic variance of the dominance effects of the homozygotes,
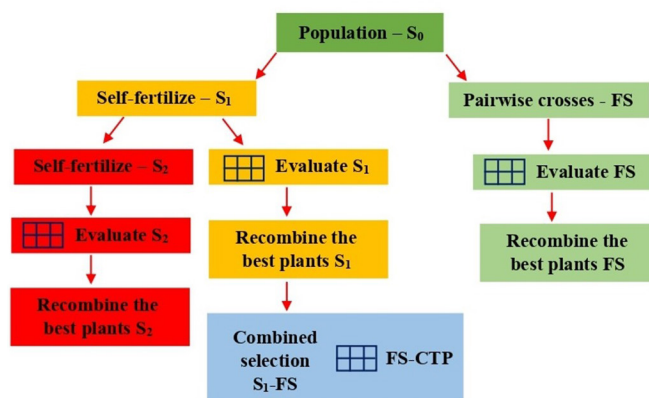

Figure 1. Diagram of the steps of recurrent selection that are being compared. The three steps of an intrapopulation recurrent selection program are presented: obtaining the progenies, evaluation, and re-combination. In addition, a proposal is made for obtaining the second cycle – S1 + FS (full-sib). In this case, the second cycle involves the cloned progeny test (CPT).

and $\breve{H}$ is the inbreeding depression squared.

Considering a locus with two alleles involved in controlling the phenotypic expression of a trait, with a frequency of the favorable allele equal to p, we have (Souza Júnior 1989):

$\sigma_A^2 = 2p(1 - p)[a + (1 - 2p)d]^2,$

$\sigma_D^2 = [2p(1 - p)d]^2,$

$D_1 = -2p(1 - p)(1 - 2p)[a + (1 - 2p)d]d,$

$D_2 = 4p(1 - p)[(1 - 2p)d]^2,$ and

$\breve{H} = [2p(1 - p)d]^2.$

As can be seen, $\breve{H} = \sigma_D^2$, and thus, $\breve{H}$ will not be considered in the estimates to be obtained. In the equation, a and d are the deviations of homozygotes and heterozygotes, respectively, from the average. The effects of these components in obtaining the estimates will be considered the same for all loci.

In obtaining information at the population level, it was considered that the allele frequencies for each locus ($0 < p < 1$) follow a beta distribution (Vencovsky et al. 2001). Under this condition, the function of density is obtained by $t_p = \Gamma(x + z + 2)\Gamma x + 1\Gamma(z + 1)px(1 - pz)$, where x and z are parameters, with $x > -1$ and $z > -1$, and Γ is the gamma function, with $\Gamma x + 1 = x\Gamma x = X!$. The average value of p in the distribution is obtained by $p = (x + 1)/(x + z + 2)$.

Six distribution functions were obtained from the beta distribution, with the following scenarios: a population that did not undergo selection, $\bar{p} = 0.2$ ($x = 1$ and $z = 7$); a population that was very minimally improved, $\bar{p} = 0.333$ ($x = 1$ and $z = 3$); one that was moderately improved, $\bar{p} = 0.4$ ($x = 1$ and $z = 2$) or $\bar{p} = 0.5$ ($x = z = 1$); and one that had already undergone some selection cycles, $\bar{p} = 0.6$ ($x = 2$ and $z = 1$) or $\bar{p} = 0.667$ ($x = 3$ and $z = 1$). From these distribution functions, the average estimates of the components of genetic variance were obtained using the following estimators:

$\bar{\sigma}_A^2 = \int_0^1 2p(1 - p)[a + (1 - 2p)d]^2 f(p)\ dp;$

$\bar{\sigma}_D^2 = \int_0^1 [2p(1 - p)d]^2 f(p)\ dp;$

$D_1 = \int_0^1 -2p(1 - p)(1 - 2p)[a + (1 - 2p)d]d\ f(p)\ dp;$

$D_2 = \int_0^1 4p(1 - p)[(1 - 2p)d]^2 f(p)\ dp.$

In the case of the full-sib (FS) progenies, the procedure used to obtain the genetic variance components was the same, remembering that only σ_A^2 and σ_D^2 occur (Table 1). For each allele frequency distribution, the following allele interactions were considered: complete dominance ($d = a$), partial dominance ($d/a = \frac{1}{2}$), and the absence of dominance ($d = 0$). The proportions of the average estimates for the six populations were obtained using the procedure adopted by Atlin and Econopouly (2022), that is, considering the total genetic variation (σ_G^2) equal to 1.0 (Table 1).

The magnitude of environmental variance (σ_E^2) was obtained from broad-sense heritabilities (h^2) at the individual level, that is, $h^2 = \dfrac{\sigma_G^2}{(\sigma_G^2 + \sigma_E^2)}$. The values for σ_E^2 were also expressed as proportions of the σ_G^2 of the population, always considered equal to 1. Under this condition, for $h^2 = 0.2$, for example, we have $\sigma_E^2 = 4\sigma_G^2$; that is, the environmental variance will be four times the genetic variance of the population. In addition to $h^2 = 0.2$, h^2 values of 0.4 ($\sigma_E^2 = 1.5\ \sigma_G^2$) and 0.6 ($\sigma_E^2 = 0.667\sigma_G^2$) were also considered.

For the evaluation of the S_1 or S_2 progenies and the FS progenies, they were considered as if the used in the design were randomized blocks with one plant per plot (single tree plots – STP). Under this condition, the mean square of the progeny source of variation (QM_p) contains $\sigma_{e*}^2 + r\sigma_p^2$. Therefore, $\bar{\sigma}_F^2 = \dfrac{QMp}{r}$, where σ_{e*}^2 is the error variance, which corresponds to $\sigma_{e*}^2 = \sigma_W^2 = \sigma_E^2 + \sigma_{GW}^2$, where σ_W^2 is the phenotypic variance among plants within the progeny, σ_E^2 is the environmental variance, and σ_{GW}^2 is the genetic variance among plants of the same progeny, that is, the genetic variance within the progenies. Note that considering STP, part of the phenotypic variance within progenies can be isolated by the effect of replications (blocks), but this was not considered. This effect is likely to be small and with limited implications for the comparisons made.

According to Souza Júnior (1989), genetic variance among progenies for S_1, disregarding the occurrence of $\breve{H}$, is $\sigma^2_{pS_1}$ = $(1)\sigma^2_A + (1/4)\sigma^2_D + (1)D_1 + (1/8)D_2$; for S_2, it is $\sigma^2_{pS_2} = (3/2)\sigma^2_A + (1/8)\sigma^2_D + (5/2)D_1 + (9/16)D_2$; and for FS, it is $\sigma^2_{pFS} = (1/2)\sigma^2_A + (1/4)\sigma^2_D$.

The genetic variance within the progenies for the S_1 and S_2 generations is:

$\sigma^2_{GWS_1} = (1/2)\sigma^2_A + (1/4)\sigma^2_D + (1)D_1 + (3/8)D_2$;

$\sigma^2_{GWS_2} = (1/4)\sigma^2_A + (1/8)\sigma^2_D + (1/2)D_1 + (3/16)D_2$;

and for FS, it is $\sigma^2_{GW_{FS}} = (1/2)\sigma^2_A + (3/4)\sigma^2_D$.

With IRS, the numerator of the expression of gain using inbred progenies contains the following (Souza Júnior 1989): $(1+ F_p)\sigma^2_A + (F_x + F_p)D_1$. In this case, F_p is the inbreeding coefficient of the generation of origin, and F_x is the inbreeding coefficient of the derived progeny. Thus, when using S_1 progenies, we have F_p=0 referring to generation S_0 and F_x =1/2. For S_2, F_p=1/2, and F_x =3/4.

Therefore, the gain from selection among (GS_A) and within (GS_w) progenies for S_1 will be:

$$GS_{AS_1} = \frac{i_A\, [\sigma^2_A + (1/2)D_1]}{\left(\frac{\sigma^2_{wS_1}}{r} + \sigma^2_{pS_1}\right)^{1/2}} = \frac{i_A\, [\sigma^2_A + (1/2)D_1]}{\left\{\frac{[\sigma^2_E + (1/2)\sigma^2_A + (1/4)\sigma^2_D + D_1 + (3/8)D_2]}{r} + \sigma^2_A + (1/4)\sigma^2_D + D_1 + (1/8)D_2\right\}^{1/2}} \quad \text{and}$$

$$GS_{WS_1} = \frac{i_w\, [(1/2)\sigma^2_A + (1/2)D_1]}{(\sigma^2_{wS_1})^{1/2}}.$$

The GS_A and GS_w progenies for S_2 will be:

$$GS_{AS_2} = \frac{i_A\, [(3/2)\sigma^2_A + (5/4)D_1]}{\left(\frac{\sigma^2_{wS_2}}{r} + \sigma^2_{pS_2}\right)^{1/2}} = \frac{i_A\, [(3/2)\sigma^2_A + (5/4)D_1]}{\left\{\frac{[\sigma^2_E + (1/4)\sigma^2_A + (1/8)\sigma^2_D + (1/2)D_1 + (3/16)D_2]}{r} + (3/2)\sigma^2_A + (1/8)\sigma^2_D + (5/2)D_1 + (9/16)D_2\right\}^{1/2}} \quad \text{and}$$

$$GS_{WS_2} = \frac{i_w\, [(1/4)\sigma^2_A + (1/4)D_1]}{(\sigma^2_{wS_2})^{1/2}}.$$

The GS_A and GS_w progenies for FS will be:

$$GS_{AFS} = \frac{i_A\, [(1/2)\sigma^2_A]}{\left(\frac{\sigma^2_{wFS}}{r} + \sigma^2_{\bar{p}FS}\right)^{1/2}} = \frac{i_A\, [(1/2)\sigma^2_A]}{\left\{\frac{[\sigma^2_E + (1/2)\sigma^2_A + (3/4)\sigma^2_D]}{r} + (1/2)\sigma^2_A + (1/4)\sigma^2_D\right\}^{1/2}} \quad \text{and} \quad GS_{WFS} = \frac{i_w\, (1/2)\sigma^2_A}{(\sigma^2_{wFS})^{1/2}}.$$

where i_A and i_W are the values of standardized selection intensities among and within progenies, respectively. These values are tabulated and depend on the percentage of plants or progenies selected. The listed value of the intensity of selection depends on the number of plants to be selected. When less than 50, it should be adjusted as proposed by Wricke and Weber (1986), that is, $i^* = i - \frac{(1 - f)}{[2if(k + 1)]}$, where i is the listed value of the standardized intensity of selection for large populations, f is the selected proportion, and k is the number of individuals being selected. All other components of the equations were previously identified.

Estimates of expected gain from IRS for the three types of progenies were obtained for the conditions already mentioned (different values of $\bar{p}$, average degrees of dominance (add) and h^2), as well as for 25 and 50 individuals per progeny.

To complete the recurrent selection cycle in the recombination, the individuals selected from the S_1 or S_2 and from FS progenies will be crossed in pairs. The mixture of the FS seeds will give rise to the first IRS cycle, for the different improvement strategies (Figure 1).

RESULTS AND DISCUSSION

The proportions of the genetic variance components, with the population σ^2_G equal to one (σ^2_G = 1) as a reference, as expected, varied with the average frequency of the favorable alleles ($\bar{p}$) and with the average degree of dominance

(add). Evidently, when dominance does not occur in the expression of the trait under selection, that is, add = 0, all the genetic variances will be additive, and $\sigma_A^2 = \sigma_G^2 = 1$ or 100% (Table 1). In the presence of dominance and p ≠ 0.5, the components σ_D^2, D_1, and D_2 arise in the inbred generations (S_1 or S_2) (Vencovsky et al. 2001). It should be emphasized that when $p = 0.5$, even with inbreeding and add ≠ zero, σ_G^2 should contain only σ_A^2 and σ_D^2. However, this did not occur because the allele frequency of 0.5 is the average of many loci that can assume any value in the distribution between 0 and 1. Additionally, under this condition, it was found that σ_A^2 decreases with an increase in the average allele frequency. Nevertheless, in all cases, it is greater than σ_D^2, D_1, and D_2, regardless of $\bar{p}$ and add (Table 1). Similar observations were made by Resende (2015) and Vencovsky et al. (2001).

As already highlighted, the proportions of σ_A^2 in relation to the other components decrease with increases in add and $\bar{p}$; however, they always remain superior to the other components (Table 1). According to Bernardo (2020), this occurs because of how the estimates of the σ_G^2 components are obtained, which is according to σ_A^2. Thus, the other components are deviations from the model, and it is hoped that they are as low as possible. Falconer and Mackay (1996) show that the proportion of σ_A^2 grows until approximately $\bar{p} = 0.667$, and the maximum σ_D^2 occurs with $\bar{p} = 0.5$ when add = 1. These results are not consistent with those obtained in the present study because, in this case, a population with the same genetic variance ($\sigma_G^2 = 1$) was always used, whereas in the estimates of Falconer and Mackay (1996), σ_G^2 is not constant: it also varies with the allele frequencies.

When one intends to use inbred progenies in intrapopulation recurrent selection (IRS) programs, a fundamental aspect is the estimate of D_1, which may have positive or negative values since it is a covariance. As D_1 in the estimator of GS is in the numerator of the expression, if it is negative, it can reduce success in selection. Table 1 shows that D_1 was negative at the lower allele frequencies and that the proportions of σ_D^2 grew with an increase in $\bar{p}$ in the population. Estimates of D_1 are not frequent in the literature. For tobacco leaf yield, the D1 estimates were negative but of small magnitude (Marques et al. 2022). Nevertheless, in common bean, the estimates of D_1, involving various traits, were negative in most cases (Souza and Ramalho 1995); this was likewise found in rice by Morais in 1992, cited by Ramalho et al. (2012). With eucalyptus, a report was found for the breast height diameter trait in which D_1 was negative, implying that the allele frequencies of the population used were low and that dominance occurred (Costa-Silva et al. 2010).

Regarding alternatives for IRS, attributing a fixed value to the σ_G^2 of the population under selection has some advantages. The first is that the result, being nondimensional, is valid for any trait. The second is that the results can be compared for different allele frequencies, degrees of dominance, and selection strategies, as performed in the present study, which considered $\sigma_G^2 = 1$. In addition, from broad-sense heritability at the individual level, the environmental variance in units of $\sigma_G^2 = 1$ can be obtained. This greatly facilitates comparisons among the selection methods. The use of a constant variance for comparing IRS strategies was also adopted by Resende and Vencovsky (1992) and Resende (2015) in eucalyptus and Marques et al. (2022) in tobacco. However, the variance adopted was based on data from experiments and considering a certain trait, which makes generalization slightly difficult. Numerous estimates of heritability have been obtained at the individual level for various traits. As expected, the values differ greatly, but they are within the interval used in this study, that is, from 0.2 to 0.6.

Table 1. Estimates (proportions) of average additive genetic variance ($\bar{\sigma}_A^2$), average dominance variance ($\bar{\sigma}_D^2$), the genetic covariance between the average additive effects of alleles and the dominance effects of the homozygotes (D_1), and the genetic variance of homozygote dominance effects (D_2) as a function of population frequency distribution. Values obtained for four populations differing in average allele frequencies ($\bar{p}$) and degree of dominance (add = d/a). Estimates considering the population with the total genetic variance equal to one ($\sigma_G^2 = 1.0$) and with inbred progenies, S_1 or S_2, and full-sib (FS) progenies

Progenies	$\bar{p}$		0.2			0.333			0.5			0.667	
	add	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
S_1 and S_2	$\bar{\sigma}_A^2$	1.00	1.06	0.94	1.00	0.98	0.83	1.00	0.88	0.67	1.00	0.75	0.44
	$\bar{\sigma}_D^2$	0.00	0.06	0.14	0.00	0.08	0.21	0.00	0.09	0.25	0.00	0.10	0.28
	D_1	0.00	-0.22	-0.33	0.00	-0.13	-0.21	0.00	-0.03	-0.08	0.00	0.08	0.06
	D_2	0.00	0.10	0.25	0.00	0.06	0.17	0.00	0.06	0.17	0.00	0.08	0.22
FS	$\bar{\sigma}_A^2$	1.00	0.95	0.87	1.00	0.93	0.80	1.00	0.91	0.73	1.00	0.88	0.62
	$\bar{\sigma}_D^2$	0.00	0.05	0.13	0.00	0.07	0.20	0.00	0.09	0.28	0.00	0.12	0.39

The expressions used in the comparisons of selection strategies were always based on the least squares method, using the denominate breeder equation (Cobb et al. 2019, Hallauer et al. 2010). Since the experiments are balanced, which is the case here, the use of mixed models does not change the obtained estimates (Bernardo 2020). When the selection is among and within progenies, it can also be performed by means of the breeder equation by adding the gains among and within (Marques et al. 2022, Resende 2002) or using what is called combined selection with best linear unbiased prediction (BLUP) (Resende 2002). For selection strategies, since phenotypic information is not available for each individual, comparisons are made using estimates of selective accuracies or heritabilities, that is, through the sum of the accuracies among plus within the progenies (Resende 2002). Thus, the inferences obtained from the two strategies must be similar in both procedures. When performing recurrent selection, a fundamental step is recombination of the superior individuals identified in the best progenies. This recombination can be performed through random pollination of the individuals/clones, by obtaining HS, or by pairwise crosses, resulted in FS. In random crosses, the efficiency of recombination is very low, especially because there may not be good synchronization in flowering. If recombination is not well performed, large parts of the time and effort dedicated to the other steps of IRS are impaired. If random crosses are used, the option would be to use a mixture of pollen and perform the hybridizations manually, as already occurs for some plants, such as tobacco. However, when performing manual hybridizations, the best option is to obtain FS, as comments by Ramalho et al. (2023), which was the procedure considered in recombination.

Although hundreds of estimates of GS have been obtained, it was not possible to present all of them. The decision was made to present the most representative cases in terms of allele frequencies, heritabilities, and average degree of dominance, making inferences of the GS for the other possibilities expected in the eucalyptus breeding programs.

Two important aspects of gain from selection are the phenotypic variances within (σ^2_W) and among (σ^2_F) progenies, since they are part of the denominator of the expressions of gain from selection within progenies (GS_W) and gain from selection among progenies (GS_A), respectively (Resende 2015, Hallauer et al. 2010). For the same heritability (h^2), the estimates of σ^2_W and the phenotypic variances among average values of progenies (σ^2_F) for the three types of progenies do not change with the average allele frequencies of the population when add = 0, for the reasons already explained above. Because of that, the estimates were placed with add = 0 only for $\bar{p}$ = 0.2 (Table 2). Notice that without the occurrence of dominance, the estimates of σ^2_W and σ^2_F are always superior to those for other scenarios with add = 1 for the same type of progeny and regardless of h^2, except for the σ^2_W estimate from FS, in which the opposite occurred.

It is clear in Table 2 that the estimates of σ^2_F vary according to the number of plants per progeny/replication (r). However, when doubling the value of r, σ^2_F decreases little. For example, with S_1, add = 1, and h^2 = 0.2, 25 plants lead to σ^2_F = 0.85, which decreases to 0.76 with 50 plants, that is, only a 10.6% reduction. Under the same conditions, however, with h^2 = 0.6, the reduction is even smaller, only 2.9%. The same is observed for the other two types of progenies.

As was commented above, with add = 0, the genetic variance of the population will be all additive, $\sigma^2_A = \sigma^2_G$ = 1. Thus, the estimate of the GS for the same h^2 will be the same for the different allele frequencies. However, as expected, the GS grew with an increase in h^2 (Table 3). For instance, with the use of S_1 progenies and add = 0, the total gain from selection (GS_T) was 21.4% greater [(2.55/2.10)/100] when h^2 = 0.6 compared to h^2 = 0.2. When using S_2 progenies for the same scenario, the advantage of greater h^2 values declines; that is, it decreases to 12.5%. With FS, the advantage of greater h^2 is more accentuated, with an increase of 30.1% [(2.03/1.56)/100]. The benefit of the increase in h^2 is more evident in GS_W than in GS_A (Table 3). For the average of the three types of progenies, with add = 0, GS_W increased by 102.9% [(0.69/0.34)/100] when h^2 = 0.6 compared to h^2 = 0.2. For GS_A, this value was only 3.7% [(1.70/1.64)/100].

The estimates of σ^2_W for FS with dominance are always superior to those obtained when S_1 and S_2 are used (Table 2). However, the estimates of GS_W for S_1 and S_2 under the same conditions of $\bar{p}$ and h^2 were always lower than those obtained with FS (Table 3). Observing the estimator of gain from selection within the progenies, it is easy to explain these results. The lowest GS_W was always obtained from S_2; this is because the numerator of the expression of gain from selection contains only $(1/4)\sigma^2_A$, and with S_1 and FS, it is $(1/2)\sigma^2_A$. In addition, when the allele frequencies are lower than 0.5, the D_1 component is negative for S_1 and S_2, reducing the expected GS. However, with p = 0.667, D_1 is not negative, and it can be concluded that the GS_W with S_1 should be greater than that with FS. This did not occur because, proportionally, σ^2_A is lower when inbreeding occurs than for FS (Table 1).

When considering GS_A, although the phenotypic variance among the average values of progenies with FS is always lower than that with S_1 and S_2 (Table 2), the GS_A for inbred progenies will always be higher than that obtained for FS (Table 3). The main reason is that the numerator of the estimator for GS_A exploits greater proportions of σ^2_A in the inbred progenies: 1 for S_1, 1.5 for S_2, and only 1/2 for FS (Hallauer et al. 2010, Marques et al. 2022). This can be better visualized by considering the average value of the GS among the six combinations of add, $\bar{p}$, and h^2 presented in Table 3. The greatest proportion of GS_T was explained by GS_A, that is, 76.7% with S_1, 88.4% with S_2, and 67.5% with FS. In the final analysis, selection among progenies is obviously more efficient than selection among individuals within them, especially for traits with low heritabilities (Table 3). These results can be explained by the h^2 of the selection units, that is, the individuals or averages of progenies. If S_1 progenies are considered, the heritability required to obtain GS_W would be $h^2_W = \dfrac{(1/2)\sigma^2_A + (1/2)\sigma^2_{D_1}}{\sigma^2_W}$. Considering add = 1, $h^2 = 0.2$, and $\bar{p} = 0.333$, using the data from Tables 1 and 2, h^2_W would be $\{[(0.5)(0.83)] + [(0.5)(-0.21)]\}/4.32 = 0.0718$. With selection among S_1 progenies, the estimator of $h^2_F = \dfrac{(1)\sigma^2_A + (1/2)D_1}{\sigma^2_F}$, and the conditions $\bar{p} = 0.333$, $h^2 = 0.2$ and r=50 lead to $h^2 = \{[(1)(0.83)] + [(0.5)(-0.21)]\}/0.78 = 0.929$, that is, a much higher value than that obtained from heritability within the progenies.

Considering different h^2 values and add = 1, the higher $\bar{p}$ is, the lower the GS_T estimate (Table 3). That is, with $h^2 = 0.2$, the average estimate of GS_T for the three types of progenies ranged from 1.75 with $\bar{p} = 0.2$ to 1.20 with $\bar{p} = 0.667$, a reduction of 30.6%. It was also found that with add = 1, $\bar{p}$ affects GS_A more than GS_W. The average estimate of GS_A for the three types of progenies with an h^2 of 0.6 was 1.07 for $\bar{p} = 0.667$ and 1.58 for $\bar{p} = 0.2$, a reduction of 32.3%. GS_W, under the same conditions, ranged from 0.38 for $\bar{p} = 0.667$ to 0.51 for $\bar{p} = 0.2$.

In recurrent selection programs, especially those involving perennial plants, it is necessary to use the time dedicated to each cycle in the best way possible. One of the options is to evaluate a large number of progenies and individuals per progeny to allow the use of high selection intensity. The results of this study confirm this (Table 4). Regardless of the type of progeny, add, and h^2, when selecting 10%, both among (A) and within (W), the GS_T exceeds that obtained with selection of 20% A and W by 25.7%. In contrast, when the intensities among and within progenies are different, a greater intensity among progenies (smaller number of progenies being selected) promotes greater gains than does a greater intensity within progenies. For example, with $h^2 = 0.2$, add = 0 and S_1, the use of 10% A and 20% W, in comparison with 20% A and 10% W, led to a 14.8% higher estimate of GS_T. Under the same conditions, for S_2 and FS, the increase in GS was

Table 2. Estimates of phenotypic variances within progenies (σ^2_W) and among the averages of progenies (σ^2_F). The reference unit is the genetic variance of the population ($\bar{\sigma}^2_G = 1$). Estimates obtained considering inbred progenies, S_1 or S_2, and full-sib (FS) progenies. Results obtained for different allele frequencies ($\bar{p}$), degrees of dominance (add), and heritabilities (h^2) with 25 or 50 plants per progeny (r)

Progenies	$\bar{p}$	add	σ^2_W	σ^2_F com $h^2 = 0.2$		σ^2_W	σ^2_F com $h^2 = 0.6$	
				r			r	
				25	50		25	50
S_1	0.200	0	4.50	1.18	1.09	1.17	1.05	1.02
		1	4.27	0.85	0.76	0.94	0.71	0.69
	0.333	1	4.32	0.87	0.78	0.99	0.74	0.72
	0.667	1	4.43	0.77	0.68	1.10	0.64	0.62
Average				0.92	0.83		0.79	0.76
S_2	0.200	0	4.25	1.67	1.58	0.92	1.54	1.52
		1	4.14	0.91	0.83	0.80	0.78	0.76
	0.333	1	4.16	1.02	0.93	0.83	0.88	0.86
	0.667	1	4.22	1.13	1.05	0.88	1.00	0.98
Average				1.18	1.10		1.05	1.03
FS	0.200	0	4.50	0.68	0.59	1.17	0.55	0.52
		1	4.53	0.65	0.56	1.20	0.52	0.49
	0.333	1	4.55	0.63	0.54	1.22	0.50	0.47
	0.667	1	4.60	0.59	0.50	1.26	0.45	0.43
Average				0.64	0.55		0.51	0.48

20.7% and 11.3%, respectively. The advantage of using a greater selection intensity, especially among progenies, is clear. Resende (2002) reports results very similar to these. However, there are few references to this in the literature. Hallauer et al. (2010) comment that the decision regarding the best selection intensity among and within progenies will depend above all on the heritability of the trait at the individual level. The smaller the h^2 is, the greater the selection intensity that should be applied among the progenies, instead of within them. This fact is quite clear from the statements above. In general, the GS decreases, as expected, with an increase in add and clearly increases with an increase in h^2 (Table 4).

The estimates of GS (Table 4) also allow a comparison of the types of progenies to be used in IRS. Taking the scenario in which, the selection intensity is 10% A and 10% W, $h^2 = 0.2$, and add = 0 as an example, it can be inferred that for S_1, the gain will be 34.6% greater than that for FS (2.10/1.56). In comparison, in the same scenario but considering $h^2 = 0.6$, the superiority of S_1 in relation to FS was slightly lower: 25.6% [(2.55/2.03)/100]. An analogous tendency occurs

Table 3. Estimates of gains from selection (*GS*) in units of population genetic variance (σ^2_G) among progenies (GS_A), within progenies (GS_W), and in total (GS_T), using S_1, S_2, or full-sib (FS) progenies. Results obtained for different allele frequencies ($\bar{p}$), degrees of dominance (add), and heritabilities (h^2). The selection intensity was 10% among progenies and 10% within progenies, considering single-tree plots (STP) and 50 replications

$\bar{p}$	add	h^2	S_1			S_2			FS		
			GS_A	GS_W	GS_T	GS_A	GS_W	GS_T	GS_A	GS_W	GS_T
0.200	0	0.2	1.69	0.41	2.10	2.10	0.21	2.31	1.15	0.41	1.56
	1	0.2	1.56	0.26	1.82	1.93	0.13	2.06	1.02	0.36	1.38
0.333	1	0.2	1.45	0.26	1.71	1.80	0.14	1.94	0.96	0.33	1.29
0.500	1	0.2	1.27	0.25	1.51	1.58	0.13	1.71	0.88	0.30	1.18
0.667	1	0.2	1.00	0.21	1.21	1.27	0.11	1.37	0.77	0.25	1.02
0.200	0	0.6	1.74	0.81	2.55	2.14	0.46	2.60	1.22	0.81	2.03
	1	0.6	1.63	0.55	2.19	2.01	0.29	2.30	1.09	0.70	1.79
0.333	1	0.6	1.51	0.55	2.07	1.87	0.30	2.17	1.02	0.64	1.66
0.500	1	0.6	1.33	0.50	1.83	1.63	0.28	1.91	0.95	0.58	1.52
0.667	1	0.6	1.06	0.42	1.48	1.31	0.23	1.54	0.83	0.48	1.31
Average			1.42	0.42	1.85	1.76	0.23	1.99	0.99	0.49	1.47

Table 4. Estimates of gains from selection in units of population genetic variance (σ^2_G), gains from selection within progenies (W), and gains from selection among progenies (A), with different selection intensities (SI) among and within, using S_1, S_2, or full-sib (FS) progenies. Results obtained considering allele frequencies ($\bar{p}$) of 0.333 and different degrees of dominance (add) and heritabilities (h^2), considering single-tree plots (STP) and 50 replications

| Progenies | SI (%) | | $h^2 = 0.2$ | | | | $h^2 = 0.6$ | | | |
| | A | W | add | | | | add | | | |
			0	%	1	%	0	%	1	%
S_1	10	10	2.10	100	1.71	100	2.55	100	2.07	100
	10	20	2.02	96	1.66	97	2.39	93	1.95	95
	20	10	1.76	84	1.42	83	2.20	86	1.76	85
	20	20	1.67	80	1.36	80	2.03	80	1.64	80
Average			1.89		1.54		2.29		1.86	
S_2	10	10	2.31	100	1.94	100	2.60	100	2.17	100
	10	20	2.27	98	1.91	99	2.51	97	2.11	98
	20	10	1.88	81	1.57	81	2.16	82	1.79	82
	20	20	1.84	79	1.54	80	2.07	80	1.73	80
Average			2.08		1.74		2.34		1.95	
FS	10	10	1.56	100	1.38	100	2.03	100	1.79	100
	10	20	1.48	95	1.31	91	1.86	92	1.65	92
	20	10	1.33	85	1.17	88	1.78	87	1.57	87
	20	20	1.24	80	1.10	79	1.62	79	1.42	79
Average			1.40		1.24		1.82		1.61	

when S_2 is compared with FS under the same conditions as above: the GS_T for S_2 is 48.1% greater [(2.31/1.56)/100] and 28.1% greater [(2.60/2.03)/100] than that for FS when h^2 = 0.2 and 0.6, respectively. In contrast, comparing S_1 with S_2 in the same context as above shows that with S_2, the gains are greater. It should be highlighted, however, that conducting each cycle of IRS with S_2 takes a longer time than with S_1 or FS.

To perform more consistent comparisons of GS, it would be important for them to be made with the same time unit. For that reason, it is fitting to highlight that all the IRS methods using progenies have three steps: obtaining the progenies, evaluation/selection of the best individuals, and recombination (Figure 1). Thus, in the comparisons made, the same time unit was considered by steps. Thus, the estimates of GS_T were obtained in accordance with the number of steps performed. With FS and S_1, in the first cycle, the number of steps is the same, three. With S_2, however, there is an additional step, which is the second self-pollination of the population to obtain the S_2 progenies; that is, there are four steps. Thus, in comparisons of the estimates of GS_T, the values for S_2 should be multiplied by ¾ to be comparable to those for S_1 and FS.

The estimates of GS_T obtained when proportionally considering the same number of steps, assuming add = 1, and varying $\bar{p}$ and h^2 are shown in Figure 2. GS_T increases with h^2 and decreases with the increase in the allele frequency of the population, regardless of the type of progeny. This implies that GS_T is quite similar when considering FS and S_2 progenies, especially with h^2 = 0.2, although the GS_T obtained with S_1 is always higher. In addition, the differences obtained when using S_1 compared to S_2 or FS magnify with an increase in h^2, especially at the lower allele frequencies of the population.

Resende and Vencovsky (1992) compare, selection among progenies of S_1 with those of HS in eucalyptus and found that the GS were always higher with S_1. Within the same scope, Resende (2002) presented results showing the relative superiority of selection among and within S_1 in relation to HS ranging from 15% when p =0.7, add = 0.75 and h^2 = 15 to 54% when p=0.7, add=1, and h^2=0.05. Coors (1999) compiled 133 results of estimates for GS in maize, some of which were based on inbred progenies. The author states that the GS with S_2 were smaller than those with S_1. Hallauer et al. (2010) also discussed results for IRS considering numerous traits and various selection methods. In the case of grain yield, they comment that the results obtained using S_1 progenies were positive only for the first selection cycle. The explanation was the rapid reduction in the variability of the population and the reduction in effective population size.

In the comparisons, the effect of effective population size (Ne) was not considered, which may have consequences for the success of IRS in the long term. The Ne for a progeny with FS is 2, that with S_1 is 1, and that with S_2 is 0.67. This is an advantage of FSs, which can be mitigated by evaluating a greater number of inbred progenies. However, in such a case, the cost of using S_1 and S_2 would be greater. It should be highlighted that there is evidence that the effective population size for long-term success with IRS does not need to be very large (Rawlings 1970). In conclusion, there need not be great concern regarding the reduction in Ne with inbred progenies.
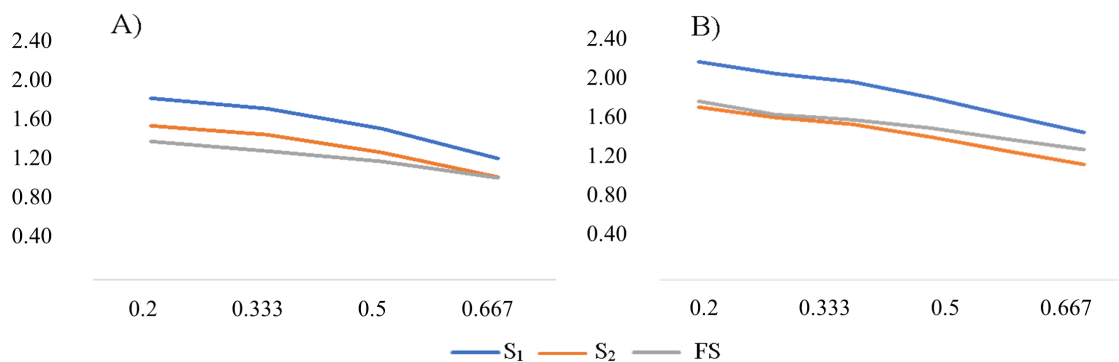

Figure 2. Estimates of total gains from selection (GS_T) per unit of genetic variance (σ_G^2), proportionally considering the same number of steps in conducting the recurrent selection program. Consideration of different inbred progenies (S_1 and S_2) and full-sib (FS) progenies, average allele frequencies of populations ($\bar{p}$), and two heritabilities (h^2) of the trait. The GS_T estimates are based on the same average degree of dominance (add = 1) and 50 plants per progeny. A) h^2 = 0.2 and B) h^2 = 0.6.

Another consideration is the possibility of S_1-FS combined selection, as has been suggested in other species (Hallauer et al. 2010, Marques et al. 2022). In the case of eucalyptus and other perennials, this approach is more promising for the following reasons: a) selected individuals from the best S_1 progenies will be recombined by crossing the plants in pairs, that is, obtaining FS progenies. Thus, the next selection cycle with FS can begin without need for the step of obtaining progenies because they were obtained during the recombination of S_1; and b) in evaluation of the FS progenies, the cloned progeny test (CPT) can be used, that is, the clones are evaluated at the same time as the FS (Resende 2002, Ramalho et al. 2021), and c) when the FS progenies are obtained from inbred plants in the case of S_1, the GS_A is greater because instead of the numerator of the equation containing 1/2 of σ^2_A, it will change to 3/4 of σ^2_A. In this situation, two more steps would be added in relation to the number S_1, that is, the evaluation of the FS and the recombination of the best individuals. Assuming that, the steps for obtaining progenies, evaluation and recombination take the same amounts of time, as already mentioned, the number of steps for each situation can be estimated, as three for S_1 or FS, four for S_2, as already mentioned, and five for S_1+ FS (Figure 1). The combined process would be superior to the others, with the additional advantage that the clones had already been evaluated, capitalizing on the genetic gains of two selection cycles (S_1 + FS). The same strategy was suggested by Resende (2002) considering the interspersed selection of S_1 with HS and emphasizes that the use of inbred progenies is desirable, as was also evidenced in the present work.

ACKNOWLEDGEMENTS

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