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Changes in DNA-marker frequencies associated with response to contrasting selection methods in *Arabidopsis*

Abstract Recurrent selection for specific combining ability (RS-SCA) and S1 family performance (RS-S1) were compared in replicated selection programs initiated from a common C0 base population of *Arabidopsis*. Three cycles of selection for aerial biomass were completed in each of two replicate programs of each selection method. Response to selection was measured on the basis of per se, S1 progeny, and testcross performance with a common tester. All selection programs improved testcross performance. Testcross gain per cycle in RS-S1 (7.15% cycle^{-1}) and RS-SCA (5.31% cycle^{-1}) were not significantly different. Performance of S1 progeny and populations per se significantly improved over cycles of selection using RS-S1 but were unchanged by RS-SCA. Codominant molecular marker-allele frequencies were recorded for each population at 22 polymorphic loci. Trends in marker-allele frequencies were tested by linear regression. Significant trends in marker-allele frequencies pooled over replicate programs were detected at 8 and 7 loci in the RS-S1 and RS-SCA programs, respectively. Marker alleles at 2 loci significantly changed frequency in response to both RS-S1 and RS-SCA programs. Marker alleles at 6 loci significantly changed frequency only in response to RS-SCA. Marker alleles at 6 other loci showed significant linear trends pooled over replicates only in RS-S1. No markers revealed increases in the frequency of different marker alleles within loci using the two selection methods. Possible genetic causes of marker frequency changes are discussed, as well as breeding implications.

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Introduction

Through selection, increases in population performance occur as a result of changes in the frequencies of favorable alleles. Many variations in recurrent selection methodologies exist and are effective at changing population performance (Hallauer and Miranda 1988). Moreover, changes in marker frequencies associated with changes in population performance have been reported (Stuber and Moll 1972; Stuber et al. 1980). However, genetic drift alone has also explained changes in gene frequency over cycles of selection (Brown 1971).

Controversy regarding the importance of additive versus nonadditive genetic effects in the expression of heterosis in hybrid maize led to the development of selection methods designed to capitalize on different types of gene action. Recurrent S1 family selection (RS-S1) is an intra-population procedure in which self-pollinated progeny are tested, and selected families intercrossed to form a new population (Fehr 1987). Selection based on S1 line performance should be effective at increasing the frequency of favorable alleles with additive and dominance effects. In contrast, recurrent selection for specific combining ability (RS-SCA) is an inter-population improvement method designed to select for overdominant gene action in testcrosses (Hull 1945). RS-SCA uses a narrow genetic base tester in the development of testcross families for evaluation. Selfed progeny of genotypes that combined best with the tester are intercrossed to develop a new population for the next cycle.

The greater importance of additive compared to nonadditive effects on yield in maize is suggested by the simultaneous improvement of both specific and general combining ability as a result of RS-SCA (Horner et al. 1973; Russell et al. 1973; Walejko and Russell 1977). In contrast, the observed response to RS-SCA in other

maize populations has been the improvement in specific combining ability alone (Eberhart et al. 1973; Sprague et al. 1959), suggesting that selection was effective at increasing the frequency of alleles with nonadditive effects that were favorable only in hybrid combination with the tester. Thus, the choice of family structure, for example, selfed or testcross, used in progeny evaluation in recurrent selection procedures may affect the value of different allelic substitutions in populations. The practical plant breeding significance is that an increase in the frequency of allelic substitutions associated with additive effects might result in selection of the best alleles for inbred performance but not the best alleles for hybrid performance, and vice versa. The magnitude of the potential compromise in hybrid performance as a result of selection for allelic substitutions with additive gene action is unknown.

In the development of F_1 hybrid cultivars in most species, plant breeders generally select for inbred performance first, to identify individuals that have accumulated favorable alleles with additive effects. Hybrid combinations are subsequently tested to exploit specific combining ability. This sequence of selection is practical, because it is often easier to evaluate inbred family performance than it is to test in hybrid combinations. Nevertheless, the correlation between inbred and testcross performance is often low, as found for yield and yield components in maize (Hallauer and Miranda 1988). Low correlations between inbred and testcross performance may be due to dominant genes in the tester masking the genetic differences among inbreds (Smith 1986) or to significant nonadditive gene action for testcross performance. If significant nonadditive gene action exists, then first increasing the frequency of alleles with favorable additive effects may preclude the later expression of certain nonadditive effects if different alleles in the population contribute to different types of gene action. Thus, selection for inbred performance prior to testcross performance may lead to the selection of alleles at some loci which would not be selected if hybrid performance were tested first.

Use of *Arabidopsis thaliana* ($2n = 2x = 10$) as a model organism in selection studies has been limited, though numerous characteristics of *Arabidopsis* make it well suited for qualitative and quantitative studies (Griffing and Scholl 1991). In the only report of recurrent selection in *Arabidopsis*, Pederson and Matzinger (1972) found low heritability and small or large dominance effects for fresh weight depending on the population examined. Heterosis has been observed in *Arabidopsis* for rosette diameter (El Ashimi and Chalbi 1976) and growth rate (Griffing and Zsiros 1970; Langridge 1962).

In the study presented here the relative responses of replicated RS-S1 and RS-SCA programs to three cycles of selection for aerial biomass were compared. *Arabidopsis* populations were used, and the primary objective was to determine with molecular markers whether the choice of contrasting RS-S1 and RS-SCA selection programs influence which loci and which marker alleles change frequency in response to selection.

Materials and methods

Selection experiments were conducted in a growth room illuminated by high-pressure sodium lamps with a photon flux density of 220–240 $\mu mol\, m^{-2}\, s^{-1}$ at soil level. The temperature ranged from 24° to 28°C when the room was maintained under continuous illumination. High temperatures ranged from 24° to 28°C during an 11-h photoperiod, and low temperatures ranged from 19° to 21°C during the 13-h dark period.

Synthesis of C0 populations

Fifteen S2 families derived from crosses among 19 inbred *Arabidopsis thaliana* ecotypes (Table 1) were crossed in a half-diallel to produce the C0 population. The C0 population was sampled twice, creating two replicate populations for each selection scheme. Each sample included 99 plants which were allowed to self-pollinate, to begin the RS-S1 study, and these were used as pollen parents in crosses to the inbred ecotype Col-wt as a tester, to begin the RS-SCA study. Thus, both testcross and S1 families were derived from the same sampled plants, giving two hybrid groups and two S1 family groups for evaluation. The first and second replicate programs of RS-S1 and RS-SCA are designated as R1S1, R2S1, R1SCA, and R2SCA, respectively.

Evaluation and selection

Families were evaluated using a simple lattice design with treatments of 99 families plus Col-wt included as a check. Five plants in a 6-cm^3 plastic pot filled with Fafard™ #2 soil mix and soaked with Peters™ 20-20-20 fertilizer at a concentration of 2 $g\, l^{-1}$ constituted

Table 1 Name, origin, and source of 19 inbred ecotypes contributing germplasm to the C0 base population

Name	Origin	Source[a]
An-1	Belgium	A.I.S. 1777
Be-0	Germany	A.I.S. 855
Ct-1	Italy	A.I.S. 2225
Cvi-0	Cape Verde Il s.	A.I.S. 2134
Eil-0	Germany	A.I.S. 2653
Est-0	Russia	A.I.S. 2074
L*er*	Poland	CrGC 9-1
Lip-0	Poland	A.I.S. 1830
Ms-0	Russia	A.I.S. 2209
Mt-0	Libya	A.I.S. 2263
Na-1	France	A.I.S. 2257
Np-0	Germany	A.I.S. 2225
Nw-4	Germany	A.I.S. 1741
Po-1	Germany	A.I.S. 1794
Sei-0	Italy	A.I.S. 2211
Ste-0	Germany	A.I.S. 2168
Sv-0	Denmark	A.I.S. 1586
Tsu-0	Japan	A.I.S. 958
Wil-2	Russia	A.I.S. 1783

[a] A.I.S., *Arabidopsis* Information Service, Frankfurt, Germany; CrGC, Crucifer Genetics Cooperative, Madison, Wis.

an experimental unit. Immediately after sowing the pots were maintained for 24 h in the growth room under continuous illumination. They were then transferred to the dark at 4°C for 24 h, after which the pots were returned to the growth room and kept under an 11-h photoperiod and a 13-h dark period until harvest. Each pot was thinned to 5 plants 9–11 days after sowing.

At 33 days after sowing, the aerial portion of plants within each plot was bulked, dried at 60°C, and weighed. Within each population evaluated, 12 families with the highest aerial biomass means, adjusted for incomplete block effects, were selected.

Population development

For both selection methods, selected S1 families were intermated in a half-diallel creating the C1 populations per se. Two to four progeny from each of the crosses of the intermating phase were grown, allowed to self-pollinate, and crossed onto Col-wt as tester. Resultant S1 and testcross families were either evaluated or stored for later use. Three cycles of each of the four recurrent selection programs were performed. One replicate population of the cycle zero RS-SCA was lost. Hence, both replicate populations of cycle one RS-SCA were derived from the same cycle zero population.

Measurement of response to selection

Composite samples of each population per se from each cycle of selection were synthesized by combining an equal number of S0 seed from each of the crosses within a given recombination block. Testcross and S1 family composites were developed by combining an equal number of seed from 100–127 sampled individuals from each population from each cycle of selection. Direct and indirect responses to selection were tested by evaluating S0, S1, and testcross plants of composite populations of all cycles of selection concurrently, as described previously. Three replications of a completely randomized block design were used in each of two replications in time. Experimental units were equivalent in size to four experimental units of the selection experiments and therefore were thinned to 20 plants each.

Competing siliques were trimmed from plants on which crosses developed, resulting in an increased seed size of S0 and testcross seed as compared to S1 seed. The weight of 1000 randomly sampled seeds from each population bulk was used as a covariate in adjusting aerial biomass to remove bias due to seed size, which is correlated with aerial biomass in *Arabidopsis* (Sills and Nienhuis 1995).

A least-squares method for comparing progress among the recurrent selection methods (Eberhart 1964) was applied to groups of populations based on their per se, inbred, or testcross genetic structure. Analyses were performed to acquire regression parameters for the response of each selection replicate as well as each selection technique pooled over replicates.

Phenotypic and additive genetic variance and narrow-sense heritability estimates for aerial biomass were calculated from variance components in each cycle of each RS-S1 replicate program. RS-SCA evaluations were not used for narrow-sense heritability estimates because allele frequencies in the tester line are not equal to that of the populations being evaluated (Empig et al. 1981). Rather, broad-sense heritabilities were calculated from estimates of genetic variance.

Population mid-parent heterosis was calculated as $[(F1\text{-}MP)/MP] \times 100$ where F1 is the average testcross performance of a given replicate of a given cycle of a given selection scheme and MP is the mean of Col-wt and the respective S0 population.

The average inbreeding coefficient based on pedigree information was calculated according to the method of Emik and Terrill (1949). Expected inbreeding coefficient, (Ft), (Falconer 1989) was also calculated via $Ft = 1/2N + (1–1/2N)Ft\text{-}1$, where t designates the cycle of selection and $2N = 24$ is the effective population size. It was assumed that $F0 = 0.0$. The effect of inbreeding in the S1 plants was calculated as $[(S1\text{-}S0)/S0] \times 100$, where S1 and S0 are the performance of given S1 and S0 populations, respectively. Regression of inbreeding effects on cycles of selection in each program was used to detect linear trends in the data.

Molecular analysis

Ecotypes Col-wt and Nd-0 were genotyped along with 19 additional inbred accessions and 147 S1 families used as parents. Marker alleles beyond those already described (Hauge et al. 1993) were determined based on mutual exclusivity of bands among the 21 accessions and the 147 S1 family parents of subsequent cycle populations. Marker-allele frequencies of populations per se were estimated based on Hardy-Weinberg theory, assuming that allele frequencies within each set of selected parental families and subsequent progeny are equal.

Isolation and treatment of plant DNA

Genomic DNA was extracted from fresh tissue of 200–300 plant bulk samples of each ecotype and family using a $2 \times$ CTAB extraction buffer (Murray and Thompson 1980).

Restriction fragment length polymorphism (RFLP) probes, hybridization, and detection

DNA from plant samples was restricted with either *Eco*RI or *Xba*I. Restriction fragments of approximately 4 μg DNA per lane were separated on 0.8% agarose gels using $1 \times$ TBE buffer (0.09 *M* Trizma, 0.09 *M* boric acid, 0.002 *M* EDTA) and transferred to neutral nylon membranes (Magnagraph, Micron Separations, Wesboro, Mass.) according to Southern (1975). DNA was bound to the membranes via UV crosslinking.

Mapped RFLPs (Hauge et al. 1993) were detected by hybridization to bacteriophage lambda clones (Chang et al. 1988). Probe DNA was generated using random primed labeling of digoxigenin-11-dUTP (Genius kit, Boehringer Mannheim, Indianapolis, Ind.). Prehybridization, hybridization, washing and detection phases were performed as described in the Genius user's guide 2.0. The prehybridization and hybridization solution contained 50% formamide, and the chemiluminescent substrate was Lumi-Phos 530™. Membranes were stripped at 100°C for 10 min in $0.1 \times$ SSC and 0.1% sodium dodecyl sulfate.

Cleaved amplified polymorphic sequence (CAPS) analysis

CAPS reactions were according to Konieczny and Ausubel (1993) except that they were performed in 10-μl glass capillary tubes in an air thermocycler (Model 1605, Idaho Technology, Idaho Falls, Idaho). Amplification conditions for the first 2 cycles were 1 min at 91°C, 1 s at 55°C, and 45 s at 72°C. The following 48 cycles were identical except that denaturation was at 91°C for 1 s. CAPS used in this study included *m429*, *GAPC*, *GL1*, *GA1*, *NCC1*, *GAPA*, *BGL1*, *AG*, and *LFY3*.

Statistical analysis

Under the null hypothesis of drift acting alone, fluctuations in marker frequencies within a selection program will be due to the sampling of parents for recombination. Marker frequency variables

follow a binomial distribution with a variance of pq/2N, where p is the frequency of one marker allele, $q = 1 - p$, and N is the number of parents recombined. In this study marker frequencies were transformed with an arcsin transformation [$\sin^{-1}$ (marker-allele frequency)$^{1/2}$] (Schaffer et al. 1977). Transformed marker frequencies have a normal distribution with variance 1/2N, independent of marker frequency.

The effect of selection versus genetic drift on marker-allele frequencies was assessed via regression. Transformed marker frequencies were regressed on cycles of selection for each selection program individually, and on pooled replicates of RS-S1 and pooled replicates of RS-SCA to test for significant linear trends by the method of Eberhart (1964). Significant changes in marker frequencies within selection programs pooled over replicates were considered as likely due to selection.

Results

Selection phase

Mean heritabilities for aerial biomass were 0.45 ± 0.17 (narrow sense) and 0.35 ± 0.17 (broad sense) for the RS-S1 and RS-SCA programs, respectively. A positive phenotypic correlation (0.22; $P < 0.05$) was observed between the performance of the S1 and testcross families derived from identical plants in the C0 population.

Average population inbreeding coefficients based on pedigree were equal to or higher in all populations than the expected inbreeding coefficients based on effective population size (Table 2).

Table 2 Average population inbreeding coefficients (F) for selection programs over cycles of selection based on pedigree and effective population size (N_e)

Cycle	Expected F[b]	Population F[a]			
		R1S1	R2S1	R1SCA	R2SCA
C_0	0.00	0.02	0.02	0.02	0.02
C_1	0.04	0.05	0.06	0.05	0.05
C_2	0.08	0.14	0.11	0.08	0.09
C_3	0.12	0.18	0.13	0.14	0.12

[a] Average F at C_n based on pedigree
[b] Expected F at C_n for $N_e = 24$

Realized gain from selection

Analysis of variance indicated significant differences in aerial biomass among composite populations, and these differences were consistent over replications in time (data not shown).

S1 and per se composite populations

RS-S1 selection resulted in increased aerial biomass for composite populations developed over cycles of selection evaluated per se and as S1 progeny (Table 3), as would be expected if genetic effects are predominantly additive for this trait. In contrast, RS-SCA selection did not change the aerial biomass for composite populations developed over cycles of selection evaluated as per se or S1 progeny.

Testcross composite populations

The indirect response to RS-S1 and direct response to RS-SCA both resulted in increased aerial biomass for composite populations developed over cycles of selection in testcross to the tester Col-wt (Table 3).

Table 3 Realized gain per cycle in milligrams per plot and as a percentage of respective predicted C_0 performance for aerial biomass of RS-S1 and RS-SCA in the per se, S1, and testcross populations, adjusted for seed size

Program		Gain per cycle[a]		
		Population		
		S1	per se	Testcross
R1S1	(mg)	60.33* ± 29.98	101.97** ± 33.02	84.58** ± 29.69
	(%)	5.31* ± 2.64	9.38** ± 3.03	8.45** ± 2.97
R2S1	(mg)	62.76* ± 31.39	35.38 ± 33.02	69.26* ± 29.31
	(%)	5.53* ± 2.76	3.24 ± 3.03	6.92* ± 2.93
Pooled S1[b]	(mg)	66.35* ± 25.58	72.94* ± 28.18	71.56** ± 24.43
	%	5.84* ± 2.25	6.68* ± 2.58	7.15** ± 2.44
R1SCA	(mg)	− 19.35 ± 29.07	10.25 ± 32.00	48.61† ± 28.44
	(%)	− 1.7 ± 2.56	0.94 ± 2.93	4.86† ± 2.84
R2SCA	(mg)	3.52 ± 29.07	6.97 ± 33.10	62.79* ± 28.44
	(%)	0.31 ± 2.56	0.64 ± 3.03	6.27* ± 2.84
Pooled SCA[b]	(mg)	− 1.59 ± 24.43	15.87 ± 27.89	53.17* ± 23.84
	(%)	− 0.14 ± 2.15	1.45 ± 2.56	5.31* ± 2.38

†,*,** Regression significant at $P < 0.10$, 0.05, 0.01, respectively
[a] Measured in milligrams per cycle ± SE and percentage of respective C_0 population ± SE
[b] Pooled response to selection for respective selection method

Heterosis and inbreeding depression

Neither heterosis nor inbreeding depression showed significant linear changes using either selection method (data not shown).

Molecular markers

Polymorphic markers

Codominant marker-allele frequencies were recorded for 28 loci. Of these loci 22 were polymorphic in the C0 population, whereas *NCC1*, *GAPA*, *BGL1*, *m326*, *AG*, and *LFY3* were monomorphic. The number of detected marker alleles per polymorphic locus ranged from two to four and averaged 2.45 in C0. The average frequency of the predominant allele within loci was 0.71. Of 54 marker alleles initially observed in the C0 population, 7 markers in the R2SCA program to 11 markers in the R1S1 program had been lost by C3. The mean number of markers observed per probe was reduced more via RS-S1 than RS-SCA (Table 4). The correlation between the inbreeding coefficient (Emik and Terrill 1949) of each cycle-program combination with its respective average number of alleles per locus was highly significantly negative ($r = -0.92$; $P < 0.01$).

Test of changes due to drift

Significant ($P < 0.05$) linear trends in marker-allele frequencies were detected in all selection programs analyzed individually. The null hypothesis that fluctuations in gene frequency are due only to genetic drift would be rejected for 5, 1, 3, and 5 of 22 loci in R1S1, R2S1, R1SCA and R2SCA, respectively.

Regression of transformed marker-allele frequencies on cycles of selection pooled over replicate programs of RS-S1 and RS-SCA provides a more robust test of whether markers at a given locus are fluctuating within the limits of drift alone. These regressions test for linear trends within each selection method. Marker alleles at 8 and 7 loci for RS-S1 and RS-SCA, respectively, had a significant linear trend in frequencies, suggesting that these or linked loci may be associated with the expression of aerial biomass in *Arabidopsis* (Table 5).

Table 4 Mean number of marker alleles observed per locus across cycles of selection for scored loci polymorphic in the base population (C_0)

Selection program	Cycle of selection			
	C0	C1	C2	C3
R1S1	2.45	2.26	1.96	1.91
R2S1	2.45	2.22	2.04	2.00
R1SCA	2.45	2.39	2.30	2.13
R2SCA	2.45	2.39	2.17	2.09

Changes in marker frequencies

Markers at each of 2 loci, *m409* and *m331*, each on different chromosomes, changed frequency in response to both RS-SCA and RS-S1 programs. Marker alleles at *m331* and 5 additional loci, *m488*, *m532*, *GL1*, *m214*, and *m217*, changed frequency in response to RS-SCA but not RS-S1 procedures. Markers that significantly increased in frequency over cycles of RS-SCA were not present in the tester, Col-wt, except in the case of *m331*. The allele present in Col-wt is always denoted by *c*. In contrast, marker alleles at loci *m241*, *m251*, *m583*, *GA1*, *m422*, and *m233* showed significant linear trends pooled over replicates only in RS-S1. No cases were detected in which the contrasting selection programs significantly favored different alleles at the same locus.

Discussion

In this study, replicated RS-S1 and RS-SCA selection programs conducted from a single base population were equally effective at increasing the aerial biomass of testcrosses, suggesting that there was no detectable compromise in hybrid performance as a result of direct selection for additive genetic effects. Selection programs evaluating inbreds, such as RS-S1, capitalize on additive genetic variance to improve populations, while those in which testcross progenies are evaluated, such as RS-SCA, will exploit additive and nonadditive genetic effects. That RS-S1 improved testcross performance suggests that additive genetic effects are important in aerial biomass accumulation in *Arabidopsis* and that these additive effects were successfully exploited by selection for inbred performance. Over the long term, RS-SCA would be expected to more fully capitalize on all types of genetic variation. In maize, S1 family selection was more effective than half-sib family selection in cycles zero to four (Burton et al. 1971; Tanner and Smith 1987), but the reverse was found in cycles four to eight (Tanner and Smith 1987).

Increases in testcross performance concomitant with population per se performance can be attributed to the covariance of additive effects of genes in testcross combination with their additive effects in the population per se (Comstock and Robinson 1956). Nonadditive effects in the overdominant range selected via RS-SCA would be expected to have limited, if any, value in the genetic background of the selected population itself. Therefore, improvements in testcross performance unmatched by population per se performance may indicate the influence of nonadditive genetic effects or the influence of inbreeding depression. In this study,

Table 5 Polymorphic marker positions, alleles, gene frequencies at cycle zero, and regression coefficients of transformed marker frequencies on cycle of selection for pooled replicates of RS-S1 and RS-SCA breeding programs

Position[a]		Locus[b]	Allele[c]	C0 gene frequency	Regression coefficient	
					Pooled-S1	Pooled-SCA
I	26.9	*m488*	*c*	0.323	0.04	− 0.02
			l	0.418	0.01	0.04*
			a	0.177	− 0.07	− 0.04*
			m	0.082	− 0.05	0.01
I	32.0	*m241*	*c*	0.247	− 0.01	0.06
			l	0.265	− 0.06	− 0.01
			m	0.417	0.07	− 0.03
			n	0.073	− 0.08*	0.06
I	56.0	*m402*	*c*	0.256	− 0.05	0.02
			l	0.549	0.01	0.00
			b	0.195	− 0.01	− 0.04
I	111.5	*m532*	*c*	0.848	− 0.03	− 0.06*
II	47.8	*m251*	*c*	0.125	− 0.12*	0.01
II	69.6	*m429*	*c*	0.256	0.03	0.00
II	81.5	*m366*	*c*	0.933	0.10‡	0.07‡
III	1.3	*GAPC*	*c*	0.79	0.12‡	0.00
III	6.6	*m472*	*c*	0.216	0.08	0.07‡
			l	0.619	− 0.03	− 0.07‡
			e	0.165	− 0.09	0.01
III	8.5	*m583*	*c*	0.412	0.10*	0.03
III	44.3	*GL1*	*l*	0.210	− 0.02	0.09**
III	72.6	*m409*	*c*	0.963	− 0.07**	− 0.05**
III	92.3	*m424*	*c*	0.223	− 0.01	0.01
IV	10.5	*GA1*	*c*	0.640	− 0.09*	− 0.05
IV	21.5	*m448*	*c*	0.332	0.00	0.02
			l	0.634	0.00	0.00
			t	0.034	− 0.04	− 0.07
IV	64.9	*m600*	*c*	0.604	0.03	0.00
			l	0.363	− 0.02	0.01
			n	0.034	− 0.04‡	–0.06
IV	72.5	*m214*	*l*	0.893	0.06	0.11*
			n	0.034	− 0.02	− 0.06
			v	0.073	− 0.08‡	–0.09*
V	10.6	*m217*	*c*	0.552	− 0.03	− 0.09*
V	20.1	*tt4*	*c*	0.732	0.01	0.02
V	55.1	*m422*	*c*	0.875	0.11*	0.00
V	73.5	*m331*	*c*	0.299	0.00	0.05*
			l	0.591	0.03	− 0.02
			e	0.110	− 0.10*	− 0.11*
V	81.9	*m233*	*c*	0.826	0.05*	0.00

[a] Chromosome and map position denoted in Roman and Arabic numerals, respectively
[b] RFLP probe or CAPS marker locus
[c] Allele symbol derived from a representative inbred parental source: *a*, *b*, *c*, *e*, *l*, *m*, *n*, *t*, and *v* from An-1, Be-0, Col-wt, Est-0, L*er*, Mt-0, Nw-4, Ct-1, and Cvi-0, respectively. Only one allele is noted for two-allele loci

testcross performance improved using both selection methods, but population per se performance improved only through RS-S1. Since inbreeding depression is not significant in these populations, the performance data suggest that nonadditive effects may have contributed to observed gains in testcross performance in the RS-SCA populations.

In this study, DNA markers were used to elucidate the allelic preferences of two contrasting selection schemes. No cases were observed in which selection via RS-S1 and RS-SCA significantly favored different alleles at the same locus. Such a situation could have serious practical significance in a breeding program, especially if alleles sub-optimal for hybrid performance are fixed in breeding populations exploited for only additive gene action, such as through RS-S1.

Differences in marker-allele frequencies between replicate selection programs initiated from the same base population are due to drift. Significant changes in marker-allele frequency over cycles of selection pooled over the replicates of each selection scheme used in this study are assumed to be due to the effects of selection. However, drift could be considerable because of the combination of a relatively high selection differential and small effective population size.

Apart from drift, other factors could result in significant changes in specific marker-allele frequency in RS-SCA but not RS-S1, as was seen at loci *m488*, *m532*, *GL1*, *m214*, *m217*, and *m331*. It is impossible to distinguish among the possible causes of differential directional marker-allele frequency changes between these selection methods.

Apparent overdominance may be caused by dominance-correlation heterosis (Houle 1989) in which genotypic correlations occur between scored loci and loci heterozygous for deleterious recessive alleles. Dominant alleles with equal coefficients of selection, s (Falconer 1989), and in repulsion-phase linkage in the base population should not change frequency in an RS-S1 program until the linkage is broken. When RS-SCA is used, one dominant favorable allele would be expected to increase in frequency if the tester is recessive for that allele and dominant for the linked favorable allele.

The overdominance hypothesis asserts that heterozygotes at single loci contribute more to performance than the respective homozygotes. Selection acting on an overdominant locus would result in the RS-S1 population changing allele frequencies to an equilibrium state dependent upon the selection coefficients of the alleles. Stuber and Moll (1972) speculated that equilibrium allele frequencies had been reached at a locus associated with grain yield in maize after nine cycles of full-sib family selection. RS-SCA would be expected to fix alleles at loci in the population complementary to the allele that is present in the tester if true overdominance is acting.

Marker alleles at *m488*, *m532*, *GL1*, *m214*, and *m217* that increased in frequency via RS-SCA but were statistically unchanged via RS-S1 were not present in the tester. With any significant contribution of overdominance to heterosis, the best cultivar will always be hybrid (Gallais 1989). Thus, selection procedures should focus on specific combining ability. In contrast, over a longer term, RS-S1 will break repulsion phase linkages and eliminate dominance-correlation heterosis.

Significant linear trends via RS-S1 but not RS-SCA for marker alleles at the *m241*, *m251*, *m583*, *GA1*, *m422*, and *m233* loci again suggest that selection acts at different loci using these selection strategies. In this case, favorable dominant alleles segregating in the RS-S1 populations would be selected for based on their additive effect. These same alleles in the RS-SCA populations would not be subject to selection if the tester was dominant at each of these loci. Thus, although the same loci contribute to performance, changes due to selection occur only through RS-S1. At the *m583*, *m422*, and *m233* loci the marker-allele present in the tester, *c*, increased in frequency in the RS-S1 populations, but not in the RS-SCA populations.

Results of this study contrast with those of Stuber et al. (1980), in which directional responses of certain alleles to selection were observed at 7 of 8 loci in both intra- and inter-population maize grain yield improvement programs. Of the marker alleles at 13 loci that significantly changed frequency in this study, only marker-allele *l* of locus *m409* increased using both types of selection. These results are consistent with the exploitation of additive genetic variance at that locus. Marker-allele *e* of locus *m331* significantly decreased across cycles of both selection methods.

Aboveground biomass in *Arabidopsis* is a quantitative trait that was significantly improved by three cycles of two contrasting recurrent selection methods. Significant selection technique-dependent trends in marker-allele frequencies were observed in replicated selection programs. Response data over cycles of selection and the molecular data suggest both additive and nonadditive genetic effects. However, no cases were observed in which RS-S1 selected for alleles that were selected against by RS-SCA, suggesting that the ultimate goal of maximally heterotic hybrids will not be precluded by breeding via RS-S1.

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