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Population-based diallel analyses among nine historically recognized alfalfa germplasms

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Abstract Identification of heterotic groups and patterns among breeding populations provides fundamental information to help plant breeders more knowledgeably manipulate heterosis. A diallel analysis was conducted among nine alfalfa (*Medicago sativa* L.) germplasms, commonly referred to as African, Chilean, Flemish, Indian, Ladak, *M. falcata*, *M. varia*, Peruvian, and Turkistan, which represent a significant proportion of the genetic diversity present in US cultivars. Heterotic responses were determined by evaluating forage yield of the germplasms and their 36 half-diallel hybrids in seeded plots that were harvested five times in each of 2 years. Commercially acceptable yields were obtained from some hybrids of unimproved parents, where at least one parent was adapted to the study environment. Variation among crosses was attributed primarily to general combining ability (GCA) effects; however, specific combining ability effects were also significant. GCA estimates for African, Chilean and Peruvian were positive, while those for Ladak, *M. falcata*, and *M. varia* were negative. Estimates for variety heterosis effects were positive for Peruvian and *M. falcata* and negative for Indian and *M. varia*. Significant mid-parent heterosis [(MPH) range of −21% to 55%] and high-parent heterosis [(HPH) range of −33% to 23%] was detected. *M. falcata* hybrids exhibited the highest MPH values. However, this likely reflects the poor yield of *M. falcata* per se in the study environment and consequently, low MPH values. Peruvian hybrids demonstrated the highest cross mean performance, significant positive MPH in all crosses, and positive HPH in five out of eight crosses. The results indicate that Peruvian should be recognized as a heterotic group. Alfalfa breeders may wish to explore opportunities for heterotic yield gains that are likely to exist in hybrids between the Peruvian germplasm and elite breeding populations, in particular, those adapted to the southwestern United States. MPH results suggest that alfalfa breeders may have capitalized on the heterotic response between Flemish and *M. varia* during past development of alfalfa synthetics adapted to the central and northern latitudes of the United States.

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Introduction

Gains in alfalfa forage yield have resulted from both the accumulation of favorable alleles and through exploitation of nonadditive genetic effects (Pfeiffer and Bingham 1983; Hill 1983; Holland and Bingham 1994; Woodfield and Bingham 1995). Evidence indicates that much of the yield gain attained since the mid-1950s likely reflects an increased emphasis on intermating between multiple alfalfa germplasms (Holland and Bingham 1994). This breeding approach was used primarily as a means to introgress more rapid regrowth into fall dormant winter-hardy populations and to incorporate resistance to multiple pests (Barnes et al. 1977).

Nine germplasm sources, commonly referred to as African, Chilean, Flemish, Indian, Ladak, *Medicago falcata*, *M. varia*, Peruvian, and Turkistan, have been recognized as primary contributors to contemporary North American alfalfa cultivars (Barnes et al. 1977). Data from a random sample of 90 cultivars and advanced experimentals registered with the North American Alfalfa Variety Review Board (http://www.naaic.org/varietyaps/lists/var&cultivar.html) during 1997–2002 indicate that some cultivars contained varying percentages of all nine germplasm sources (Table 1). In most cases, one to four

germplasms (primarily two) usually predominated. The intermating of these germplasms decades ago undoubtedly resulted in linkage disequilibrium and subsequently, heterosis in many cases. However, multiple cycles of recurrent selection have since been practiced in these materials. Concern has been expressed that yield genes in these genetically diverse populations may now be approaching equilibrium (Barnes et al. 1977; Holland and Bingham 1994). This could hinder yield progress by limiting future opportunities to capitalize on heterosis through intermating of diverse gene pools for short-term yield increases. Riday and Brummer (2002) reported that alfalfa yield improvement appears to have slowed appreciably in some areas of the United States since the early to mid-1980s.

Following strategies that resulted in the tremendous success of the hybrid maize industry (Melchinger and Gumber 1998), it is clear that future efforts must be directed towards identifying heterotic groups in alfalfa. Population improvement efforts must be practiced within each group to maintain each population's genetic integrity. Subsequent hybridization between groups will provide breeders with the chance to exploit many forms of epistasis and to repeatedly capture heterosis (Bingham 1983). Opportunities to utilize heterosis in alfalfa as a means to increase forage yield has been nicely summarized by Brummer (1999) and demonstrated (Busbice and Rawlings 1974; Riday and Brummer 2002).

Information describing population genetic parameters of these nine key germplasms may provide insight for developing future breeding strategies to continue improving alfalfa yield in North America. The present study was conducted to: (1) estimate genetic effects for these nine germplasms in order to provide retrospective insight into the magnitude of additive and nonadditive effects that have contributed to yield increases during the past 50 years, (2) evaluate their respective diallel hybrids to identify potential heterotic combinations that may still exist, and (3) document the usefulness of employing unimproved germplasms in alfalfa breeding programs as a means to immediately capitalize on heterosis.

Materials and methods

Accessions representing each of nine alfalfa germplasm sources: African, Chilean, Flemish, Indian, Ladak, *M. falcata*, *M. varia*, Peruvian, and Turkistan (Barnes et al. 1977), were evaluated in this study. The *M. falcata* germplasm (*M. sativa* subsp. *falcata*) was represented by 30 genotypes derived from a single genetically broad-based accession, WISFAL (Bingham 1993). The other eight germplasms (*M. sativa* subsp. *sativa* and subsp. *varia*) were represented by 15 plants from each of two "relic" seed lots that were produced on or before 1960. Relic accessions were used to increase the chance of germplasm purity, because out-crossing and contamination of more recently derived populations may have had a greater chance of occurring as alluded to by Barnes et al. (1988) and Kidwell et al. (1994). The germplasms and their representative accessions were African (Moapa and Higazzi); Chilean (Arizona Chilean and Chilean 21-5); Flemish (DuPuits and Alfa); Indian (FC23631 and FC32174); Ladak (Ladak 1 and Ladak 2); *M. varia* (Grimm and Cossack); Peruvian (Hairy Peruvian and Coastal Hairy Peruvian); and Turkistan (Samarkand and Tokmak). Additional information on the populations used in this study has been provided elsewhere (Segovia-Lerma et al. 2003). The nine germplasms were intermated by hand (without emasculation) in a half-diallel to produce 36 F_1 hybrids. The F_1 populations were generated by crossing each plant within a germplasm to one other plant in each of the other eight germplasms. Parental populations were synthesized by randomly intercrossing all genotypes within a given germplasm. An equal number of seed, within each cross from each plant, was bulked to form balanced composite populations for each inter- and intracross population.

The 36 F_1 crosses, the nine parents, and four check cultivars (Dona Ana, Wilson, Commercial 1, and Commerical 2) were planted during March 1996, using a randomized complete block design with three replications. Each population was planted in three-row plots, 1.5 m long, and seeded at a rate of 300 seed plot^{-1}. Rows within plots were spaced 30 cm apart, and plots were spaced 60 cm apart. Control plots of the cultivar Dona Ana were established between the entry plots to minimize interplot interactions. Plots were seeded on a Glendale sandy clay loam (Typic Torrifluvent, pH 8.0) at the Leyendecker Plant Science Research Center near Las Cruces, N.M., USA. Prior to planting, plots were fertilized with 122 kg ha^{-1} of phosphorous, and no additional fertilizer was applied to the plots after establishment. Plots were irrigated every 14 days from 15 April to 15 October during 1996–1998. No data were collected during the 1996 establishment year. Plots were harvested five times, at 30-day intervals, during May–September of 1997 and 1998, using a flail harvester that cut at a 5-cm height. Forage yield data

Table 1 Percentage contribution of nine germplasms to alfalfa cultivars and elite experimental lines (n=90) released in the United States during 1997–2002

Region of adaptation	n	African	Chilean	Flemish	Indian	Ladak	*M. falcata*	*M. varia*	Peruvian	Turkistan	Unknown
North Central and Northeast	48	0	5	40	0	9	5	22	1	12	6
Northwest	17	1	6	42	2	6	3	22	1	8	9
Great Plains	9	0	17	42	0	3	4	18	1	12	3
Southwest	16	38	9	5	19	2	1	4	6	13	3

(kg ha^{-1}) were adjusted to a dry-matter basis by subsampling approximately 300 g of fresh forage from each plot and drying it at 60°C for 48 h. Seasonal forage biomass production was determined by summing the yield data over harvests within each year.

Heterogeneity in soil texture resulted in spatial variation within replicates. The data were adjusted for field trend effects, using nearest neighbor analysis via the "second-difference approach" (Besag and Kempton 1986; Stroup et al. 1994), as provided by Agrobase Software (Agronomix Software, Portage la Prarie, N.B., Canada). Analysis of variance of adjusted seasonal biomass data across years was that of a split plot in time, considering entries as the whole-plot factor and years as the split-plot factor. Analysis of biomass production in each year was also conducted. The data were analyzed using two different approaches. To determine the performance of the experimental populations relative to the four checks, a standard analysis of variance was conducted that ignored the diallel arrangement. When considering the entry diallel arrangement, the check cultivars were excluded, and entries were partitioned into parents/varieties and crosses. Crosses were partitioned into heterosis, average heterosis, variety heterosis, general combining ability (GCA), and specific combining ability (SCA) according to analyses II and III of Gardner and Eberhart (1966). Hypotheses of particular interest between parent and cross means were examined using the "general linear hypothesis" approach as described by Murray et al. (2003).

Table 2 shows formulas of the estimates of diallel effects that were of particular interest in this study, in terms of sample and population means. Analysis II was used to estimate the effects of heterosis, average heterosis, and variety heterosis, but was not used to provide variety effect estimates, which were confounded with GCA effects (Murray et al. 2003). Analysis III was used to estimate variety/parental and GCA effects. SCA from analysis III is identical to analysis II "specific heterosis" (Murray et al. 2003). Diallel effect estimates were obtained by using PROC GLM (SAS Institute 1989). The standard error of the diallel-effect estimates and their significance (i.e., different from zero) were obtained using a *t*-statistic generated by PROC MIXED (SAS Institute 1992). The effects of varieties (i.e., parents), heterosis, average heterosis, variety heterosis, GCA, and SCA were estimated based on contrasts between parent and cross means via ESTIMATE statements in GLM or MIXED. Each contrast (C) was constructed as a linear function of the plot observations:

$$C = \sum_{j=1}^{t} c_j \bar{y}_j$$

where c represents the contrast coefficients subject to $\sum c_j = 0$, and $\bar{y}_j$ represents the sample mean of entries (i.e., parents or crosses), and t denotes the number of entries involved in the contrast.

Mid-parent heterosis (MPH) and high-parent heterosis (HPH) were estimated according to Hallauer and Miranda (1988) and expressed as:

$$\mathrm{MPH} = 100[\mathrm{F}_1 - \{(\mathrm{P}_1 + \mathrm{P}_2)/2\}]/\{(\mathrm{P}_1 + \mathrm{P}_2)/2\}$$

$$\mathrm{HPH} = 100[(\mathrm{F}_1 - \mathrm{HP})/\mathrm{HP}]$$

where F_1 is the performance of the hybrid obtained by crossing parents P_1 and P_2, and HP is the highest yielding parent in a given cross. The significance of heterosis effects was estimated using a Student *t*-test (α=0.05):

$$t = L/\sqrt{(\mathrm{MSE}rk)\left(\sum_{j=1}^{t} C_j^2\right)}$$

where L is the respective contrast estimate of absolute heterosis (i.e., MPH or HPH); r is the number of replicates; k is the number of years; MSE is the mean square error of the whole-plot (i.e., entry × block mean square) obtained in the analysis of variance of parents and

Table 2 Estimates of effects tested in a diallel among nine alfalfa germplasms. *GCA* General combining ability, *SCA* specific combining ability

Source[a]	Parameter	Estimate[b]
Varieties (parents)	v_j	$\bar{y}_{jj} - \bar{y}_v$
Heterosis	$H_{jj'}$	$\bar{y}_{jj'} - \frac{1}{2}\left(\bar{y}_{jj} + \bar{y}_{j'j'}\right)$
Average heterosis	$\bar{h}$	$\bar{y}_c - \bar{y}_v$
Variety heterosis	h_j	$\frac{n-1}{n-2}\left[\sum_{j'=1, j\neq j'}^{n} \frac{\bar{y}_{jj'} - \frac{1}{2}\left(\bar{y}_{jj} + \bar{y}_{j'j'}\right)}{n-1} - \bar{h}\right]$
GCA	g_j	$\frac{n-1}{n-2}\left(\bar{y}_{jc} - \bar{y}_c\right)$
SCA/heterosis	$s_{jj'}$	$\bar{y}_{jj'} + \frac{n}{n-2}\bar{y}_c - \frac{n-1}{n-2}\left(\bar{y}_{jc} + \bar{y}_{j'c}\right)$

[a]Effects for variety (i.e., parents) and GCA effects estimated according to analysis III of Gardner and Eberhart (1966). Effects for heterosis, average heterosis, variety heterosis estimated according to analysis II of Gardner and Eberhart (1966). SCA as estimated by analysis III was comparable to that of specific heterosis as estimated by analysis II.

[b]$\bar{y}_{jj}$ sample mean of parent/variety j, $\bar{y}_{jj'}$ sample mean of cross of parents j and j', $\bar{y}_v$ sample mean of all parents/varieties, $\bar{y}_c$ sample mean of all crosses of all n parents, $\bar{y}_{jc}$ sample mean of crosses from parent j. μ_{jj}, $\mu_{jj'}$, μ_v, c_j, and μ_{jc} are the corresponding population parameters.

crosses (i.e., 45 entries); and $\sum_{j=1}^{t} C_j^2$ is the sum across entries (i.e., parents and crosses) of the squared contrast coefficients estimating the respective heterosis effect. The contrast coefficients were defined based on the contrast linear structure as described above for estimating diallel effects.

Results and discussion

Relative performance of parents and diallel hybrids

Parent effects were highly significant as demonstrated by a tenfold range in the mean forage yields between *M. falcata* and Chilean (Table 3). No parent population yielded as well as the best check (Wilson). African and Chilean exhibited the best parental performances per se and yielded similarly to the lower three checks. Contrasts (α=0.05) between the average yield of the four checks and that of the nine parent germplasms classified the germplasms into four performance categories: (1) Chilean and African; (2) Indian, Peruvian, and Turkistan; (3) *M. varia*, Flemish, and Ladak; and (4) *M. falcata*.

Commercially acceptable yields were obtained from some hybrids where at least one parent was adapted to the study environment. Ten of the 36 hybrids produced forage yields comparable to the mean of the four checks (Table 3). Each of these ten hybrids was derived from crosses involving either the Peruvian or Chilean parents. The strong performance of Chilean and its hybrids was expected in our study environment based on previous experience (Ray et al. 1998). Three hybrids, African × Peruvian, Chilean × Peruvian, and Chilean × Flemish, yielded similarly to Wilson (the best check).

Analysis of variance across years, based on analyses II and III (Gardner and Eberhart 1966; Murray et al. 2003), indicated that all diallel effects significantly influenced dry matter yield (Tables 4, 5). The results were similar to those obtained from analyses conducted within each year. With the exception of average heterosis, all main effects demonstrated a significant interaction with years. The magnitude of the interactions, however, was considerably less than that of their respective main effects. Parents differentially transmitted genetic effects influencing forage yield to their progeny, as demonstrated by the significance observed for heterosis, average heterosis, variety heterosis, GCA, and SCA effects. The GCA mean square was considerably larger than that of SCA, indicating that additive effects were of major importance. The significance of the heterosis, average heterosis, variety heterosis, and SCA effects indicate that opportunities also exist to capitalize on hybrid vigor associated with specific population crosses.

Estimates of genetic effects

Ranking of the nine parents based on their performance per se was identical to that of analysis III variety effects (Table 6). Six shifts in rank occurred when parental performance was compared with that of analysis II variety effects (data not shown). This demonstrates that parent and GCA effects are confounded within analysis II variety effects as reported by Murray et al. (2003). Estimates of GCA effects were significant and positive for Peruvian, Chilean, and African. Conversely, *M. falcata*, *M. varia*, and Ladak exhibited significant negative effects. GCA effects for Flemish, Indian, and Turkistan were not significantly different from zero.

Estimates for variety heterosis effects were significantly greater than zero for Peruvian and *M. falcata*, and less than zero for Indian and *M. varia* (Table 6). Three of the four positive SCA effects were detected in dormant × nondormant or dormant × semidormant hybrids, indicating that important alleles/allelic combinations influencing forage yield can be obtained using materials generally considered to be unadapted to a given region. Two of these four hybrids (African × Peruvian and Chilean × Flemish) ranked no. 1 and no. 3 for yield, and performed as well as the best check. Busbice and Rawlings (1974) reported that forage yield of hybrids generated between Chilean and

Table 3 Dry matter yield (kg ha^{-1}) of parents (*diagonal*) and crosses (*above diagonal*) from a diallel set of nine alfalfa germplasms averaged over two years (ten harvests) and three replicates

Germplasm	African	Chilean	Flemish	Indian	Ladak	*M. falcata*	*M. varia*	Peruvian	Turkistan	Mean
African	8,627	8,150	7,487	8,110	8,104	7,208	7,631	10,614	8,217	8,190
Chilean		8,635	9,594	8,692	8,586	7,361	8,367	10,498	8,851	8,762
Flemish			6,180	7,838	5,484	4,766	7,017	8,803	7,723	7,339
Indian				7,982	5,379	5,429	6,673	8,793	7,641	7,319
Ladak					5,607	4,936	5,694	8,326	7,043	6,694
M. falcata						857	4,236	6,262	5,527	5,716
M. varia							6,445	8,042	6,904	6,820
Peruvian								7,412	8,973	8,789
Turkistan									7,337	7,610
Mean (of four checks)										9,578
Range (of checks)										9,012–10,677
LSD $_{(0.05)}$										1,250

Table 4 Mean squares for dry-matter yield (kg ha^{-1}) from a diallel analysis among nine alfalfa germplasms within and across 2 years (ten harvests), conducted according to analysis II of Gardner and Eberhart (1966). This table reflects a revised analysis II (as described by Murray et al. 2003) and emphasizes partitioning of variation for heterosis

Source	*df*	Mean squares		
		1997	1998	Across years
Blocks (B)	2	81,829	11,187	70,929
Entries (E)	44	7,564,303**	11,992,792**	18,528,370**
Parents/varieties (*V*)	8	32,277,068**	52,668,642**	82,752,394**
Heterosis (*H*)	36	2,072,577**	2,953,714**	4,256,365**
Average heterosis($\bar{h}$)	1	19,298,107**	16,259,171**	35,492,228**
Variety heterosis (*h*)	8	3,488,824**	4,000,160**	6,864,039**
Specific heterosis (*sh*)	27	1,014,966*	2,150,862**	2,326,837**
E × B	88	543,683	732,205	997,596
Years (Y)	1			5,570,209**
E × Y	44			1,028,724**
V × Y	8			2,193,316*
H × Y	36			769,926**
$\bar{h}$ × Y	1			65,050
h × Y	8			624,945*
sh × Y	27			838,990**
Residual	90			272,599
CV %		10.3	11.5	7.2

Significance levels: *P=0.05; **P=0.01

Flemish accessions were comparable to commercial checks and numerically superior to all other hybrids generated among eight groups of alfalfa introductions. These results emphasize that significant yield improvements can be attained by exploiting nonadditive effects among unimproved populations. A significant SCA effect was not detected for the second-highest yielding hybrid (i.e., Chilean × Peruvian). This likely reflects the fact that Chilean and Peruvian possessed the highest GCA estimates in our study, and that the GCA of each parent was essentially removed during the estimation of the SCA effect associated with a particular cross (Table 2). Historical records and molecular analyses of the nine germplasms also indicate that Chilean and Peruvian share a distant but common ancestry (Barnes et al. 1977; Kidwell et al. 1994; Segovia-Lerma et al. 2003). SCA effects were significant and negative for the African × Chilean, Flemish × Ladak, Indian × Ladak, and *M. falcata* × Peruvian hybrids.

The importance of GCA in contributing towards alfalfa forage yield has been documented here and elsewhere (Dudley et al. 1969; Groose and Bingham 1988; Hill 1983; Busbice and Rawlings 1974). While SCA was significant in our study, its contribution was less than that of GCA. This may have occurred because the SCA associated with individual genotypes involved in a cross

Table 5 Mean squares for dry-matter yield (kg ha^{-1}) from a diallel analysis among nine alfalfa germplasms within and across 2 years (ten harvests) conducted according to analysis III of Gardner and Eberhart (1966)

Source	*df*	Mean squares		
		1997	1998	Across years
B	2	81,829	11,187	70,929
E	44	7,564,303**	11,992,792**	18,528,370**
V	8	16,104,356**	18,672,734**	34,144,998**
Varieties vs crosses($\bar{h}$)	1	19,298,107**	16,259,171**	35,492,228**
Crosses (C)	35	5,277,839**	18,344,851**	14,474,173**
General combining ability (GCA)	8	19,661,535**	37,996,068**	55,471,434**
Specific combining ability (SCA)	27	1,014,966*	2,150,862**	2,326,837**
E × B	88	543,683	732,205	997,596
Years (Y)	1			5,570,209**
E × Y	44			1,028,725**
V × Y	8			632,092*
$\bar{h}$ × Y	1			65,050
C × Y	35			1,146,917**
GCA × Y	8			2,186,169**
SCA × Y	27			838,990**
Residual	90			272,599
CV %		10.3	11.5	7.2

Significance levels: *P=0.05; **P=0.01

Table 6 Estimates of diallel effects for varieties/parents (v), GCA, ($\bar{h}$),h, SCA, and their respective standard errors (in *parentheses*) for alfalfa dry-matter yield (kg ha^{-1}) over five harvests in each of 2 years

Effect[a]	Estimate	Effect	Estimate	Effect	Estimate	Effect	Estimate	Effect	Estimate	Effect	Estimate
v_{11}	2,063[c]	GCA_{11}	822[c]	h_{11}	−209	SCA_{12}	−1618[c]	SCA_{27}	164	SCA_{48}	−11
v_{22}	2,070[c]	GCA_{22}	1476[c]	h_{22}	440	SCA_{13}	−655	SCA_{28}	45	SCA_{49}	185
v_{33}	−384	GCA_{33}	−151	h_{33}	41	SCA_{14}	−10	SCA_{29}	−254	SCA_{56}	359
v_{44}	1,418[c]	GCA_{44}	−174	h_{44}	−882[c]	SCA_{15}	699[b]	SCA_{34}	691	SCA_{57}	−145
v_{55}	−958[b]	GCA_{55}	−888[c]	h_{55}	−409	SCA_{16}	921[c]	SCA_{35}	−948[c]	SCA_{58}	237
v_{66}	−5,708[c]	GCA_{66}	−2006[c]	h_{66}	848[c]	SCA_{17}	82	SCA_{36}	−548	SCA_{59}	301
v_{77}	−120	GCA_{77}	−744[c]	h_{77}	−683[c]	SCA_{18}	815[b]	SCA_{37}	440	SCA_{67}	−486
v_{88}	848[b]	GCA_{88}	1506[c]	h_{88}	1082[c]	SCA_{19}	−235	SCA_{38}	−23	SCA_{68}	−709[b]
v_{99}	772[b] (380)	GCA_{99}	159 (143)	h_{99}	−227 (238)	SCA_{23}	797[b]	SCA_{39}	245	SCA_{69}	−96
						SCA_{24}	−81	SCA_{45}	−1031[c]	SCA_{78}	−191
						SCA_{25}	527	SCA_{46}	138	SCA_{79}	18
μ_v	6565	$\bar{h}$	906[c] (150)			SCA_{26}	420 (349)	SCA_{47}	119 (349)	SCA_{89}	−163 (349)

[a]Diallel effects of parent germplasms designated as *11* African, *22* Chilean, *33* Flemish, *44* Indian, *55* Ladak, *66 M. falcata*, *77 M. varia*, *88* Peruvian, *99* Turkistan. SCA designations such as "12" reflect the SCA of the hybrid between African (11) and Chilean (22), etc.
[b,c]Effects are significantly different from 0 at α=0.05 and 0.01, respectively

was diluted by the compositing of seed over multiple genotypes within each cross. In addition, SCA is not easily detected in autotetraploid diallels (Dudley et al. 1969). Busbice and Rawlings (1974) also detected significant SCA effects in crosses between eight diverse groups of alfalfa. SCA effects were not significant, however, in the studies of Dudley et al. (1969), Hill (1983), or Groose and Bingham (1988). Upon examining the pedigree of the populations involved in these studies, we observed that SCA effects were detected only when multiple divergent and genetically distinct populations were used to generate hybrids. SCA effects were not significant in studies where hybrids were produced using parents from "related" and/or highly recombined germplasm (Dudley et al. 1969; Hill 1983; Groose and Bingham 1988). This observation suggests that in order for alfalfa breeders to optimize heterotic response, future hybridization efforts must utilize genetically distinct populations, many of which likely reside outside of conventional breeding populations.

Heterosis among hybrids

HPH ranged from 23% in the African × Peruvian hybrid to −33% for the Ladak × Indian hybrid (Table 7). MPH ranged from 55% in the Chilean × *M. falcata* hybrid to −21% in the Indian × Ladak hybrid. Parents ranked similarly for their variety heterosis (Table 6) and mean MPH values (Table 7). Six crosses exhibited significant positive values for both MPH and HPH, implicating the expression of dominant or partially dominant alleles linked in repulsion and/or complementary epistasis. Other crosses demonstrated only significant positive MPH. The only hybrid demonstrating significant positive MPH between the dormant populations, Flemish, Ladak, and *M. varia* was Flemish × *M. varia*. Even though these three germplasms were not well adapted to our study environment, this observation is interesting, given that most elite populations adapted to northern latitudes of the United States contained primarily Flemish and *M. varia* germplasm (Table 1). Apparently, breeders capitalized on this heterotic response in the past to develop alfalfa synthetics.

Hybrids involving *M. falcata* as a parent exhibited the highest MPH values. Our results, those of Riday and

Table 7 High-parent [(HPH) *above diagonal*] and mid-parent [(MPH) *below diagonal*] heterosis for forage yield from diallel crosses among nine alfalfa germplasms

Germplasm	African	Chilean	Flemish	Indian	Ladak	*M. falcata*	*M. varia*	Peruvian	Turkistan	HPH mean
African		−6	−13*	−6	−6	−17*	−11*	23*	−5	−5
Chilean	−5		11*	1	−1	−15*	−3	21*	3	1
Flemish	1	30*		−2	−11*	−23*	9	19*	5	−1
Indian	−2	5	11*		−33*	−32*	−17*	10	−4	−10
Ladak	14*	21*	−7	−21*		−12*	−12*	12*	−4	−8
M. falcata	52*	55*	35*	23*	51*		−34*	−15*	−25*	−22
M. varia	1	11*	10	−8	−6	15*		8	−6	−8
Peruvian	32*	31*	30*	14*	28*	52*	16*		21*	12
Turkistan	3	11*	14*	0	9	34*	0	22*		−2
MPH mean	12	20	16	3	11	40	5	28	12	

Brummer (2002), and molecular data presented by Kidwell et al. (1994) and Segovia-Lerma et al. (2003) support the premise that *M. falcata* represents a distinct heterotic group. However, the high MPH in our study likely reflects the exceptionally poor yield of *M. falcata*, and consequently, low MPH values. Given *M. falcata*'s strong negative GCA effect (Table 6) and its exceptionally low HPH (average −22%), considerable effort would be required to advance this heterotic group (e.g., reduced fall dormancy, improved pest resistance, and heat tolerance) to a point where it might be useful as a parent in our study environment (a strategy not unlike that proposed by Brummer 1999).

Hybrids involving Peruvian as a parent showed the highest cross mean performance (Table 3), significant MPH in all crosses, and significant positive HPH in five out of eight crosses (Table 7). Peruvian also possessed a GCA effect comparable to that of Chilean (Table 6), indicating its value as a parent for breeding purposes. Townsend et al. (1994) and Barnes et al. (1977), however, reported that Peruvian germplasm has been utilized in only a small percentage of US alfalfa varieties and its contribution to those varieties is small (<10%). Our results and molecular evidence provided by Kidwell et al. (1994) suggest that Peruvian should also be recognized as a heterotic group.

Conclusions

High yields and HPH were obtained for several unimproved alfalfa hybrids in our study. Hybridization between elite materials and unimproved heterotic partners, therefore, may offer a useful short-term approach to immediately exploit alfalfa yield gains via heterosis. Commercial release of Syn1 hybrids is not yet a practical approach to capture maximum heterosis in alfalfa cultivars. Brummer (1999) demonstrated that, in most cases synthetic hybrids in random mating equilibrium should have higher frequencies of triallelic and tetraallelic genotypes than their respective parents. Thus, one would postulate that the advancement of a synthetic hybrid through generations of seed increase for commercial release (i.e., to the Syn3 or Syn4) should still produce a population that is superior to either parent. Care must be exercised when considering the best approach to develop synthetic hybrids. Mixing seed of both parental populations to produce "chance hybrids" is perhaps the easiest approach. However, if random crossing is not attained during generations of seed increase, the expression of heterosis may never be fully realized (Brummer 1999). If "pure hybrid" seed were to be generated by hand, however, followed by planting of the Syn1 seed for breeder and advanced generations of seed increase, opportunities to capture heterosis should be improved. Given the importance of GCA effects in influencing alfalfa forage yield, opportunities should also exist to continue selection within the hybrid population itself. During the selection process within the hybrid population, however, parental alleles may be lost. Thus, maintaining original heterotic parent populations would still be warranted to help conserve parent alleles.

Evaluation of additional accessions within germplasm repositories (e.g., the National Plant Germplasm System) is also warranted to identify populations with greater heterotic potential than those reported here. Given Peruvian's ability to generate highly heterotic hybrids with most of the germplasms evaluated, it is possible that genetically broad-based, commercial breeding populations may demonstrate significant heterosis if hybridized to Peruvian. The utility of this approach is being evaluated using hybrids developed between a number of elite populations/cultivars that each contain a significant proportion of one of the nine germplasms described herein (I. M. Ray 2003, unpublished data). Results of that work will be reported elsewhere.

The development of a high-yielding and multiple pest resistant Peruvian population, as an elite heterotic group, appears to have merit (particularly for use in the southwestern United States). If winterhardy nondormant heterotic gene pools are to be developed, as suggested by Brummer (1999), the Peruvian and potentially Chilean germplasms may be particularly useful heterotic sources for hybridization to elite cultivars that are adapted to the Northern latitudes of the United States. During a germplasm conversion process, it is crucial that each heterotic source be subjected to population improvement strategies that maintain its genetic integrity (e.g., intrapopulation recurrent selection for yield and backcrossing of pest resistance traits). This approach will ensure that future yield improvements can continue to be made through selection for favorable additive alleles. Subsequent hybridization between heterotic groups will allow breeders to capitalize on nonadditive forms of genetic variation and epistasis in order to repeatedly capture yield gains through heterosis.

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