Chapter Two

Yield and Fertility of Populations Representing the Introgression of *Medicago sativa* Germplasm into Diploid Alfalfa

Introduction

I. Yield and Fertility

Cultivated alfalfa (*Medicago sativa* L.) exists naturally at the tetraploid level (2n=4x=32) (McCoy and Bingham, 1988). Fully fertile, seed propagated populations of cultivated alfalfa at the diploid level (CADL) were produced for research purposes (Bingham and McCoy, 1979). In this study, CADL was used to address the question: how will genes adapted to tetraploidy perform at the diploid level and how will they interact with genes derived from the naturally diploid wild relative of alfalfa (*M. falcata*)?

CADL was produced by haploidizing tetraploid *M. sativa* (Bingham, 1969). The original haploids were male sterile, however, such that a diploid *M. sativa* population could not be seed propagated. These haploids had some female fertility, so they were used as female recurrent parents in a backcrossing scheme with diploid *M. falcata*. After 7 generations of backcrossing, a fully fertile population containing approximately 100% cultivated germplasm was produced. The backcrossing series consisted of populations ranging from 0 to

100 percent of cultivated germplasm. Such a set of populations containing differing levels of wild and cultivated genes was used in this study to investigate the interaction of wild and cultivated germplasm at the diploid level.

Since the genome of M. sativa is adapted to the tetrasomic condition, diploidized M. sativa may be at a disadvantage compared to the naturally diploid M. falcata. Mac Key (1970) suggested that allogamous polysomic polyploids will depend greatly on genic interactions and dosage effects of multiple alleles. At the diploid level, however, no multiple allelic interactions are possible, such that vigor must be determined by additive, dominant, and epistatic gene effects alone. In addition, a large genetic load can be maintained in M. sativa because of tetrasomy and diploid (1n=2x) gametes. Such a large frequency of deleterious recessive alleles would be exposed at the diploid level.

Fox (1981) compared responses to inbreeding in isogenic diploid and tetraploid alfalfa populations. It appeared that inbreeding depression in tetraploid alfalfa was mainly due to loss of interactions between multiple alleles or linkage groups. Inbreeding depression in isogenic diploid populations, on the other hand, was due simply to increased homozygosity. Thus, quantitative, multigenic traits such as yield and fertility appear to be controlled differently in diploids as compared to tetraploids.

Groose et al. (1988) compared general and specific combining abilities between isogenic diploid and tetraploid alfalfa populations. They found that there was no correlation between a genotype's performance at the diploid level and its isogenotype's performance at the tetraploid level. They concluded that, "some alleles, linkage blocks and epistatic interactions that have positive effects at one ploidy level may have negative effects at the other."

Self-pollinated progeny of diploids have 50% less heterozygosity than their parents and exhibit severe inbreeding depression (Fox, 1980 and Ray, 1989). This inbreeding depression is attributable to the increased frequency of homozygous recessive loci in inbred populations (Ray, 1989).

Diploid S_1 populations are more dependent on favorable allele frequencies and less dependent on heterosis than are their more heterozygous S_0 parents. Therefore, S_1 populations of diploids with varying percentages of wild and cultivated germplasm can be compared to determine which populations have the higher frequencies of favorable alleles for yield at the diploid level.

II. 2n Gametes

2n gametes produced by diploid relatives of alfalfa have played an important role in germplasm transfer (Bingham,

1968). 2n gametes may also be important in future breeding efforts. 2n pollen formation in diploid alfalfa is due to disoriented spindles at metaphase II of meiosis, effectively a first division restitution mechanism (Vorsa and Bingham, 1979). The resultant gametes are highly heterozygous and can be used to maximize heterozygosity in progeny of 4x-2x crosses (Bingham, 1983, Peloquin, 1983, McCoy and Rowe, 1986). 2n egg formation in alfalfa is due to lack of proper cytokinesis following the second meiotic division (Pfeiffer and Bingham, 1983), equivalent to a second division restitution mechanism. The resultant 2n eggs are not highly heterozygous and several sexual polyploids produced via 2n eggs must be used to transfer the maximum number of alleles to a population. Importantly, 2n eggs in combination with 2n pollen allow for bilateral sexual polyploidization. In this case, a 2x-2x cross results in 4x progeny (Peloquin and Mendiburu, 1972).

2n pollen production is controlled by a single recessive gene with variable expressivity (McCoy, 1982). The genetic control of 2n egg production is not clearly understood, although it appears to be independent of 2n pollen production (Veronesi et al., 1986). Plants producing 2n pollen and/or 2n eggs have been found in _M. falcata_, _M. coerulea_ (a very close diploid relative of _M. sativa_) and CADL populations (Bingham and McCoy, 1979 and Veronesi et al., 1986). Veronesi et al. (1986) screened 274 diploid plants from 1 CADL population, 2

M. falcata populations, and 5 *M. coerulea* populations for 2n gamete production. Populations varied for percent of plants producing 2n pollen (0-51%) and 2n eggs (46-100%) as well as for average seed set following 4x-2x or 2x-4x crosses (0-30%). All populations contained some plants that produced at least one type of 2n gamete. Also, plants within each population varied in terms of 2n gamete production.

With the CADL-*M. falcata* backcross series, populations differing in percent of wild and cultivated germplasm can be screened for 2n gamete production. Populations producing high or low levels of 2n pollen or 2n eggs can then be identified. On the one hand, populations that produce high levels of 2n gametes will be useful for transferring germplasm between ploidy levels and maximizing heterozygosity via interploidy crosses. On the other hand, populations that produce low frequencies of 2n gametes will be useful as stable diploid populations needed for research purposes.

Materials and Methods

Yield Study:

Eight diploid alfalfa populations representing a range of percentages of cultivated tetraploid *Medicago sativa* germplasm in diploid form were used in the yield trial: 2x WISFAL-1, 70-21, 72-24, 73-20, 76-20, 1985 CADL, IVR CADL, and PC CADL (Table 1). 2x WISFAL-1 is a diploid population derived by intercrossing 22 *M. falcata* plant introductions and advancing four generations by intercrossing survivors in source nurseries from 1970 to 1985. 1985, IVR, and PC CADLs all contained 100% *M. sativa* germplasm, yet differed in terms of amount of selection and type of *M. sativa* germplasm. 1985 CADL was derived from 25 plants of populations advanced from 76-20 by adding germplasm from 15 *M. sativa* haploids between 1978 and 1984. IVR CADL was derived from an intercross of 25 selected plants from a population produced by crossing three triploids of cultivar Iroquois S_3 X 1985 CADL and three Vernal S_3 X 1985 CADL triploids with 6 selected 1985 CADL plants. PC CADL was derived from an intercross of 25 selected plants from a population produced by crossing 10 selected IVR CADL plants with 10 haploids of WTKW (an experimental tetraploid *M. sativa* cultivar selected during inbreeding).

Approximately 25 plants of each population were dug from the field during November, 1989 and transplanted to the

Table 1. Diploid populations used in the study.[1]

	Germplasm	
Population	% *M. sativa* (cult.)	% *M. falcata* (wild)
2x WISFAL-1	0.00	100.00
70-21	50.00	50.00
72-24	75.00	25.00
73-20	87.50	12.50
76-20	96.87	3.13
1985 CADL	100.00	0.00
IVR CADL	100.00	0.00
PC CADL	100.00	0.00

1 See Bingham and McCoy, 1979.

greenhouse. S_0 generations of a given population were produced by randomly intercrossing the plants in that population and collecting as balanced a bulk as possible of the seeds. S_1 generations of a given population were produced by self-pollinating the 25 plants by tripping the flowers with a spatula lacking any foreign pollen and by collecting as balanced a bulk as possible of the seeds. All seed was produced in the greenhouse during January to March, 1990.

Seeds were germinated in soil in April and May, and in peat pots in late May, 1990. Plants were transplanted from soil to a site at the northern end of field 385 at Arlington, WI on June 27, 1990. Plants were transplanted from peat pots to the East end of field 382 at Arlington, WI on August 1, 1990. The experimental design was a split block in space and time. Successive harvests were made on the same plots over time. Whole units in space were generations of inbreeding, while subunits were populations. Experimental units were 2 meter rows of 5 plants spaced 0.5 meter apart. Rows were spaced 1 meter apart. Sampling units were individual plants. The experiment was replicated four times in field 385 and six times in field 382. Units were missing from some reps because not all populations produced enough plants for equal replication.

Field 385 was harvested once for total herbage on October 12, 1990. Fresh weights were not measured, but the entire

forage yield of each plant was dried at 60^{0}C and total dry weights were measured. Field 382 was not harvested during 1990.

Both sites were harvested on June 6, 1991. Total dry weights were measured for each plant. Percent survival of plants was measured on each plot as the number of plants producing any herbage as of June 6, 1991 out of the total number originally planted in the plot. Data were analyzed using SAS procedures GLM and CORR. Data were tested for, and met, the assumptions underlying the general linear model. Years, locations, and replications were considered to be random effects. Populations and levels of inbreeding (generations) were considered to be fixed effects.

Reproduction Study:

Approximately 25 plants of each of the populations used in the field study were transplanted to the greenhouse for seed production in November, 1989. Each plant was measured for self- and cross-fertility, and 2n pollen and 2n egg production.

Self-fertility was measured by tripping approximately 50 flowers per plant with a spatula free of foreign pollen. Selfed racemes were tagged, and seeds were harvested when mature. Self-fertility was measured as number of seeds produced per selfed flower.

Cross-fertility was measured by tripping flowers on approximately 2 racemes per plant with a spatula carrying a sample of pollen from each plant in the population. These inter-crossed racemes were tagged, and seeds were harvested when mature. Cross-fertility was measured as number of seeds produced per intercrossed flower.

2n pollen production was assayed by pollinating one raceme per pollinator of a tetraploid white flowered male-sterile plant with pollen from each diploid plant in the study. Test-cross racemes were tagged and seed was harvested when mature. Any seed produced from such a cross must have originated in one of three ways: as a hybrid between a 2n=2x pollen grain from the 2x pollen parent and a 1n=2x egg from the 4x maternal parent, as a rare self of the tetraploid parent if male-sterility does not exhibit completely expressivity, or as a haploid. Assuming androgenetic haploids are unlikely, self and gynogenetic haploid progeny would be white-flowered and male sterile. Only the progeny derived from 2n pollen would have pigmented flowers and would shed pollen. Therefore, all seeds produced from 4x-2x testcrosses were germinated in peat pots, transplanted to soil, and screened for flower color and pollen production. Chromosome counts from root tip squashes were used to confirm ploidy level where necessary. 2n pollen production was measured as number of hybrid tetraploid plants produced from this 4x-2x

cross per flower pollinated.

2n egg production was assayed by allowing each plant in the study to be open-pollinated with a mixture of 1x and 2x pollen in the field. Plants were transplanted to the field at Madison, WI in April, 1990. Plants were spaced 1m apart in rows, with 2x and 4x plants alternating within each row. A bee box was placed in the field to allow pollen transmission via leafcutter bees (_Megachile_ _rotundata_ F.). Plants were allowed to open-pollinate for the entire summer of 1990. Total seed from each plant was harvested in September, 1990. For each plant, a row containing a sample of its open-pollinated seed was sown at the Arlington field May 13, 1991. These rows were screened for tetraploid progeny, which must have resulted from a mating of a 2n=2x egg from a diploid plant with a 1n=2x pollen grain from a tetraploid plant, on July 12, 1991. Plant ploidy level was estimated based on the size and shape of leaves, stems, and flowers. Because the sample size of progeny from each plant differed greatly, a numerical assay was not attempted. Rather, each plant in the original experiment was classified as a 2n egg producer or non-2n egg producer based on the presence or absence of tetraploids in its open-pollinated progeny rows.

Data were analyzed using the SAS procedures GLM, CORR, and NPAR1WAY. Data were tested for the assumptions underlying the general linear model. All data sets met the assumptions

except for the data from the 4x-2x testcross. This data contained predominantly zero values, and was highly skewed from normality. In order to accommodate this, the nonparametric Kruskal-Wallis test was used. Additionally, the values were converted to ranks and the ranks were analyzed with SAS procedure GLM, which gave an identical result.

Results

Because individual plants were harvested, it was possible to identify which plants survived transplanting in order to produce herbage yield. Thus, the yield data could be analyzed in two ways. First, the plants that did not survive until harvest could be measured as producing zero yield. A second method would be to ignore the dead transplants and analyze the yield data of surviving plants only.

Because only one of the two locations was harvested in both years of the experiment, the linear additive model of the experiment cannot include both harvests and years. The interaction between locations and years, which would be included in such a model, cannot be measured. Therefore, in order to determine the location effect, the data from 1991 alone was analyzed using a model involving locations (Table 2). This analysis considered dead plants as zero yielders. The effect of locations was not significant in this analysis, so location effects were ignored in all other analyses.

Table 3 presents the results of analyzing the yield data including dead plants with locations combined. The very small ($p = .9515$) effect of experimental error (error b) indicated that there was no evidence that experimental error was greater than sampling error (plants/plots). Therefore, the two error terms were pooled for F tests. Inbreeding levels

Table 2. Analysis of variance for diploid yields at two locations in 1991 (dead plants = 0 yield).

Source	df	Mean Square	F value	p-value
Locations	1	1241.187	0.26	.6251
Reps/Loc	8	4808.884	3.32	.0021
Generations	1	96145.630	14.42	.1639
Loc*Gen	1	6665.891	3.98	.0812
Error (a)	8	1675.707	1.16	.3329
Populations	7	26734.546	12.85	.0016
Loc*Pop	7	2052.130	1.42	.2069
Gen*Pop	7	7283.693	1.47	.3123
Loc*Gen*Pop	7	4959.222	3.42	.0025
Error (b)	100	1448.496	0.93	.6788
Plants/Plot	583	1565.036		

Table 3. Analysis of variance for diploid yields, locations combined (dead plants = 0 yield).

Source	df	Mean Square	F value	p-value
Replications	9	4817.327	1.63	$p > .250$
Generations	1	119562.563	31.10	$p < .001$
Rep*Gen	9	3872.133	2.40	$p > .250$
Populations	7	24006.336	28.09	$p < .001$
Gen*Pop	7	8825.068	3.48	$p < .005$
Error (a)	114	2059.740	1.21	$.05 < p < .10$
Years	1	9675.740	3.27	$p > .010$
Rep*Years	3	2957.647	1.74	$p > .010$
Gen*Years	1	24.749	0.02	$p > .250$
Rep*Gen*Years	3	1616.099	0.94	.4226
Pop*Years	7	854.687	0.50	.8385
Gen*Pop*Years	7	968.690	0.56	.7877
Error (b)	41	1138.226	0.66	.9515
Plants/Plots	831	1726.294		
Pooled Error	872	1698.644		

(generations) and populations, as well as the interaction between the two, were all highly significant effects.

The mean yields of populations at both levels of inbreeding are given in Table 4. 2x WISFAL-1 can be considered the control because it is naturally diploid. Relative to 2x WISFAL-1, no consistent trends can be described for these populations. For example, population 70-21 performed significantly better than 2x WISFAL-1 as an S_0 population, but significantly worse as an S_1 population. Neither the percent of *M. sativa* germplasm or amount of selection that have gone into a population can adequately explain these yield performances.

S_0 and S_1 yields were plotted against percent of *M. sativa* germplasm in each population in Figure 1. The line graphs indicate no simple relationship between yield and percent of *M. sativa* germplasm.

Absolute and percent inbreeding depressions of each cultivar are presented in Table 5. The ranges in values are tremendous: from 21.9% to 75.2%. 2X WISFAL-1 had the lowest percent inbreeding depression, which was approximately half that of the population with the next lowest inbreeding depression. Population 70-21, which had the highest S_0 yield, also had the highest inbreeding depression.

In order to determine the effect on yield of differential survival of populations, percent survival was analyzed (Table

Table 4. Mean yields of diploid populations at two levels of inbreeding (dead plants = 0 yield).

Population	S_0 Yield[1]	Population	S_1 Yield
70-21	94.19 a[2]	2x WISFAL-1	55.90 a
2x WISFAL-1	71.59 b	1985 CADL	33.38 b
1985 CADL	57.20 c	73-20	25.10 c
76-20	46.21 d	70-21	23.38 cd
73-20	45.33 d	IVR CADL	19.92 cde
IVR CADL	35.28 e	76-20	18.65 de
72-24	30.61 ef	PC CADL	15.17 ef
PC CADL	28.23 f	72-24	12.36 f

1 Grams dry weight.

2 Means within an inbreeding level followed by the same letter are not significantly different using a .05 probability level LSD.

Figure 1. Introgression of M. sativa germplasm into diploid alfalfa.

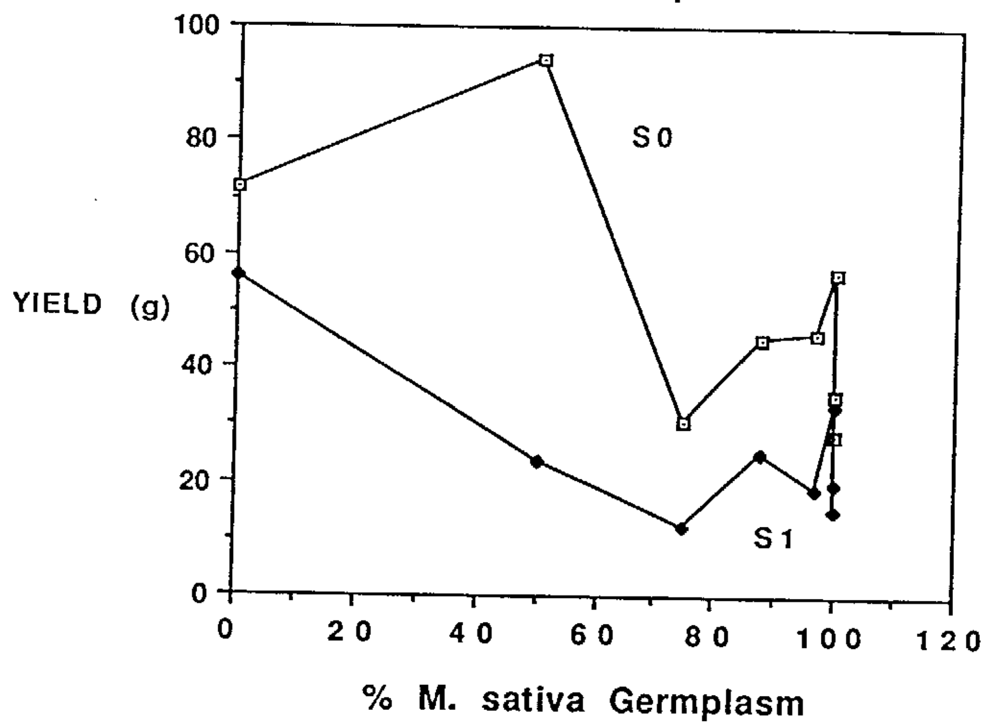

Table 5. Inbreeding depression of diploid populations.

Population	Inbr. Depr.[1]	Population	% Inbr. Depr.
72-24	12.36	2x WISFAL-1	21.9
PC CADL	13.06	72-24	40.4
IVR CADL	15.36	1985 CADL	41.6
2x WISFAL-1	15.59	IVR CADL	43.5
73-20	20.23	73-20	44.6
1985 CADL	23.82	PC CADL	46.3
76-20	27.56	76-20	59.6
70-21	70.81	70-21	75.2

1 Inbreeding depression in grams dry weight.

6). Locations had the most significant effect ($p=.0018$) on survival, most likely because one location was not harvested in 1990, and therefore was under less stress. Populations also showed a significant ($p = .0448$) effect on percent survival. Population rankings for percent survival (Table 7) closely resembled the rankings for yield. This indicates that there is the possibility that population yields may have been strongly influenced by percent survival. Populations with low survival rates would have had their mean yields skewed downwards by many plants that produced zero yield.

In order to evaluate population yields without having percent survival as a confounding factor, yields were analyzed on the basis of surviving plants only. Table 8 presents this analysis of variance. Again, populations, inbreeding levels, and their interaction were all significant. The population yields based on surviving plants only are given in Table 9. The absolute yields of all populations were increased, of course, by ignoring dead plants. The relative performances of populations, however, did not differ greatly whether non-surviving plants were considered or not (compare Tables 4 and 9). In either case, no simple trends can be identified to explain the yield performances.

The analysis of variance for self-fertility of the diploid populations (Table 10) indicates that there were highly significant ($p = .0001$) differences between

Table 6. Analysis of variance for percent survival of diploid plants at two locations.

Source	df	Mean Square	F value	p-value
Locations	1	1.41602	20.97	.0018
Reps/Loc	8	0.06754	1.85	.0763
Generations	1	0.81844	52.16	.0876
Loc*Gen	1	0.01569	0.65	.4448
Error (a)	8	0.02429	0.67	.7205
Populations	7	0.17630	3.96	.0448
Loc*Pop	7	0.04449	1.22	.2992
Gen*Pop	7	0.03927	1.15	.4293
Loc*Gen*Pop	7	0.03415	0.94	.4826
Error (b)	101	0.03650		

Table 7. Percent survival of diploid plants at two levels of inbreeding.

Population	S_0 % surv.[1]	Population	S_1 % surv.
70-21	90.0	2x WISFAL-1	80.0 a[2]
2x WISFAL-1	90.0	73-20	76.0 a
1985 CADL	85.0	76-20	76.0 a
76-20	84.0	IVR CADL	72.5 ab
IVR CADL	80.0	1985 CADL	70.0 ab
73-20	73.3	70-21	62.0 abc
PC CADL	72.0	PC CADL	50.0 bc
72-24	71.5	72-24	42.5 c

1 There are no significant differences between population means at the S_0 level.

2 Means followed by the same letter are not significantly different using a .05 probability level LSD.

Table 8. Analysis of variance for surviving plant yields of diploid populations.

Source	df	Mean Square	F value	p-value
Replications	9	5878.822	3.29	p > .10
Generations	1	85129.371	16.77	p < .005
Rep*Gen	9	4011.657	3.00	p > .10
Populations	7	18493.993	31.65	p < .001
Gen*Pop	7	6116.678	2.54	p < .025
Error (a)	7	2287.208	1.32	p = .01
Year	1	1368.802	0.77	p > .25
Rep*Year	3	1788.345	1.03	p > .25
Gen*Year	1	1142.913	0.86	p > .25
Rep*Gen*Year	3	1336.370	0.77	p > .25
Pop*Year	7	584.274	0.34	p > .25
Gen*Pop*Year	7	804.193	0.46	p > .25
Error (b)	41	986.461	0.55	p = .990
Plants/Plot	593	1789.357		
Pooled Error	634	1737.435		

Table 9. Yields of surviving plants at two levels of inbreeding.

Population	S_0 Yield[1]	Population	S_1 Yield
70-21	101.44 a[2]	2x WISFAL-1	66.32 a
2x WISFAL-1	78.30 b	1985 CADL	43.27 b
1985 CADL	63.70 c	70-21	34.10 bc
73-20	58.12 c	73-20	33.15 bc
76-20	53.25 cd	IVR CADL	28.37 c
72-24	43.36 de	PC CADL	37.29 c
IVR CADL	43.32 de	72-24	23.92 c
PC CADL	37.29 e	76-20	23.74 c

1 Grams dry weight.

2 Means within an inbreeding level followed by the same letter are not significantly different using a .05 probability level LSD.

Table 10. Analysis of variance and mean performances for self-fertility of diploid populations.

Source	df	Mean Square	F value	p-value
Populations	7	0.39176	11.48	.0001
Plants/Pop	180	0.03414		

Mean self-fertilities of diploid populations.

Population	Self-fertility[1]
1985 CADL	0.4112 a[2]
76-20	0.2018 b
PC CADL	0.1033 bc
2x WISFAL-1	0.0930 c
74-20	0.0926 c
70-21	0.0642 c
73-20	0.0400 c
IVR CADL	0.0231 c

1 Seeds produced per selfed flower.

2 Means followed by the same letter are not significantly different using a .05 probability level LSD.

populations. Table 10 also lists the self-fertilities of each population. Again, there seem to be no consistent trends to explain the results. 2x WISFAL-1 is ranked in the middle of the populations containing M. sativa germplasm. There also seemed to be no consistent trend between a population's yield performance and self-fertility. 1985 CADL was the best-yielding CADL population and it also had the highest self-fertility. On the other hand, PC CADL, which was a very low-yielding line also had a relatively high (.1033) self-fertility, and 70-21 which performed well as an S_0 population had a very low self-fertility.

Analysis of variance for cross-fertility (Table 11) indicates that populations differed significantly for cross-fertility as well. Mean population cross-fertilities listed in Table 11 again present no apparent trends. 2x WISFAL-1 had the highest cross-fertility, while 1985 CADL had the second highest and IVR CADL had the lowest. Apparently, percent of M. sativa germplasm is not the major factor controlling fertility. The only consistent result has been that 1985 CADL appeared to be a relatively vigorous population in terms of yield as well as self- and cross-fertilities.

The results of the 2n gamete screening indicate that populations were not significantly different in terms of 2n gamete production. Table 12 presents an analysis of variance of the ranks of 4x-2x testcross performances, which is

Table 11. Analysis of variance and mean performances for cross-fertility of diploid populations.

Source	df	Mean Square	F value	p-value
Populations	7	12.86096	10.01	.0001
Plants/Pop	178	1.28499		

Mean cross-fertilities of diploid populations.

Population	Cross-fertility[1]
2x WISFAL-1	2.660 a[2]
1985 CADL	2.325 ab
76-20	2.021 ab
70-21	1.722 bc
72-24	1.107 cd
PC CADL	1.103 cd
73-20	0.906 d
IVR CADL	0.605 d

1 Seeds produced per flower cross-pollinated.

2 Means followed by the same letter are not significantly different using a .05 probability level LSD.

Table 12. Analysis of variance of 4x-2x testcross performance ranks.

Source	df	Mean Square	F value	p-value
Populations	7	1470.9430	0.94	.4782
Plants/Pop	179	1567.4575		

equivalent to a Kruskal-Wallis nonparametric test of the values themselves. Either analysis indicates that populations did not differ significantly in terms of 2n pollen production. Table 13 summarizes the data from the 2n gamete screening. Both 2n pollen and 2n eggs were produced at low frequencies in each population studied. Only population 73-20 appeared to contain plants producing higher levels of 2n pollen. A chi-square goodness of fit test indicates that the number of 2n pollen producers that also produced 2n eggs was not significantly different from that expected if the two traits were completely independent.

Table 13. 2n gamete production of diploid populations.

Population	mean 2n pollen production[1]	no. plants producing 2n pollen[2]	no. plants producing 2n eggs[3]
2x WISFAL-1	0.0081	3/23	2/23
70-21	0.0162	8/25	3/25
72-24	0.0120	3/21	1/21
73-20	0.4114	6/23	1/23
76-20	0.0082	3/22	1/22
1985 CADL	0.0425	8/24	2/25
IVR CADL	0.0137	5/26	1/24
PC CADL	0.0261	6/24	4/23
Mean	0.0646	0.223	0.080

1 4x seeds per pollination of tetraploid male sterile tester.

2 Number of plants producing tetraploid progeny via 4x-2x cross.

3 Number of plants producing tetraploid progeny via open-pollination with tetraploid pollinators.

	no. of 2n pollen producers that also produce 2n eggs	
Expected	3.387	
Observed	5	$p > .10$

Discussion

The yield performances of the populations used in this study defy simple explanation. 2x WISFAL-1 was the best yielding population at both levels of inbreeding, except for 70-21, which had the highest S_0 yield. Since 2x WISFAL-1 is the naturally diploid population, it serves as the standard against which the other populations can be compared. The superior S_0 yield of 70-21 can be explained on the basis of heterosis. A hybrid population containing 50% wild germplasm and 50% cultivated germplasm would be expected to exhibit great heterosis due to the combination of these two very unrelated germplasms. The severe (75%) inbreeding depression of this population supports this idea. If 70-21's heterosis was due to complementary positive combinations of *M. sativa* and *M. falcata* genes, many of these combinations would be destroyed by inbreeding, resulting in severe inbreeding depression.

2x WISFAL-1 had the lowest percent inbreeding depression of the populations studied. Its percent inbreeding depression was at most only about one half of the percent inbreeding depression of any of the populations containing *M. sativa* germplasm. This can be explained on the basis of a smaller genetic load present in 2x WISFAL-1 compared to the other populations. However, this is opposite of the conclusion

reached in the tetraploid yield study of this thesis. In that case, 4x WISFAL-1 had the greatest percent inbreeding depression of any cultivar at the tetraploid level. The only way to reconcile these findings at the present time is to conclude that alfalfa germplasm that is not at its natural ploidy level tends to exhibit more severe inbreeding depression than germplasm that has evolved at that same ploidy level. It is not clear why this should be so.

All populations containing *M. sativa* germplasm had significantly poorer yields compared to 2x WISFAL-1, with the exception of 70-21 at the S_0 level. This may be an indication that gene action is very different at the tetraploid level compared to the diploid level. Even following selection, the pure *M. sativa* CADL populations were not competitive with 2x WISFAL-1. In fact, the amount of selection involved in CADL populations was inversely related to their yields. PC has undergone more selection than IVR, which has had more selection than 1985 CADL. At both levels of inbreeding, 1985 CADL out-yielded IVR, which out-yielded PC CADL in turn. This cannot be explained simply as a narrowing of the germplasm base during selection for more vigorous types, because the S_1 population yields have decreased with selection. It is not clear why this has occurred. No simple relationship appears to exist between a population's level of selection or *M. sativa* germplasm and its yield performance.

The results of the fertility study are similarly confusing. 2x WISFAL-1 had the highest cross-fertility of any population, as might be expected. However, 2x WISFAL-1 fell in the middle of the rankings in terms of self-fertility. There seems to be no relationship between a population's level of selection or percent of cultivated germplasm or yield and its self- or cross-fertility. The 1985 CADL population appeared to be the only population with consistently vigorous responses for each trait. Ignoring 2x WISFAL-1, it was the best population in terms of S_1 yield, self- and cross-fertility, and the second best for S_0 yield.

The results of the 2n gamete production study are at least comprehensible. Each population contained some few plants that produced low levels of 2n pollen and some few plants that produced 2n eggs. The distribution of 2n gamete producing plants appeared to be random across populations. These traits are apparently not affected by the germplasm source of the population or selection on the basis of normal fertility. Also, the fact that there was no correlation between 2n pollen and 2n egg production supports the conclusion of Veronesi et al. (1986) that the two traits are under separate genetic controls.

Those plants identified as high-level 2n pollen producers in this study have been combined as the parents of a new experimental population. This is an attempt to produce a

diploid population that contains many plants that produce high levels of 2n pollen. Such a population will be useful for future studies of 2n gametes and as an aid in germplasm transfer between *M. falcata* and *M. sativa*.

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APPENDIX

Selection of HK CADL Population

The latest population of CADL plants was selected out of the populations used in the diploid study of this thesis plus two extra populations: Delta D and a second sample population of PC CADL, PCB (Table 1). Delta D is a diploid population derived from triploids containing cultivar Vernal germplasm. Individual plants were selected on the basis of high seed set, uprightness, vigor, purple flowers, and tightly coiled seed pods. Plants were selected in the field at Madison, WI in July, 1990 and transplanted to the greenhouse in September, 1990. Plants were then hand intercrossed to form the first population of HK CADL. One parent plant in the population, from the 76-20 population, produced undesirable black seeds, and therefore was excluded from further intercrosses in order to keep black seed genes out of the HK CADL population.

Table 1. Sources of HK CADL parent population.

Population	# plants selected	% *M. falcata*
72-24	1	25.00
73-20	1	12.50
76-20	4	3.13
1985 CADL	1	0.00
IVR CADL	1	0.00
PC, PCB CADL	4	0.00
Delta D	7	0.00
Total	19	2.61