Growth Analysis of *Medicago sativa ssp falcata* and *Medicago sativa ssp sativa* at two ploidy levels.

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ABSTRACT

The morphology, forage quality and productivity of a diploid and tetraploid populations of *Medicago sativa ssp falcata* (2*x* and 4*x* WISFAL-1) and *M. sativa ssp sativa* (W2xiso-1 and W4xiso-1), were compared in a single trial at Arlington, Wisconsin between 1989 and 1991. Changes in 4*x* WISFAL-1 morphology relative to 2*x* WISFAL-1 were consistent with those expected with elevated ploidy including increased leaf size, flower size, and plant height. 4*x* WISFAL-1 also produced nearly 30% more DM than 2*x* WISFAL-1, and with its faster regrowth is better adapted to a three-cut system than the diploid *M. falcata* population. 4*x* WISFAL-1 has potential for alfalfa improvement and its ability to cross directly with cultivated alfalfa allows unlimited gene transfer from *M. falcata.*

INTRODUCTION

Cultivated alfalfa (*Medicago sativa ssp sativa* L.) and *M. sativa* ssp *falcata* (L.) Archangeli (hereafter referred to as *Medicago sativa* and *Medicago falcata*) both have naturally occurring diploid and tetraploid populations (Clement, 1962; Bingham, 1975; Barnes et al., 1977; Lesins and Lesins, 1979). Gene transfer between *M. sativa* and *M. falcata* at the same ploidy level occurs freely (Stanford et al., 1972; McCoy and Bingham, 1988), while the presence of unreduced (*2n*) gametes facilitates gene transfer between ploidy levels (Ledingham, 1940; Stanford et al., 1972; Vorsa and Bingham, 1979; Bingham and Saunders, 1974; Bingham et al., 1991; McCoy and Bingham, 1988; Veronesi et al., 1986). Genes can also be transmitted from higher to lower ploidy levels through haploidy, thus completing a flexible diploid-polyploid-haploid reproductive system in *Medicago* (Bingham, 1971; Bingham and Saunders, 1974; Bingham et al., 1991).

M. falcata has played a unique role in alfalfa improvement. Introgression of *M. falcata* genes into cultivated alfalfa has contributed winterhardiness, disease resistance, and hybrid vigor (Barnes et al., 1977). Most of the early alfalfa cultivars with adaptation to colder North America regions (e.g. 'Grimm', 'Ladak', 'Cossack' and 'Rambler') contained *M. falcata* germplasm either from direct hybridization with *M. falcata* or from *Medicago varia* (Martyn), the naturally occurring hybrid between *M. falcata* and *M. sativa*. Benchmark cultivars such as 'Narragansett', 'Iroquois' and 'Vernal' contained more than 25% *M. falcata*, and have subsequently contributed to many modern cultivars (Barnes et al., 1977; Bingham et al., 1991).

M. falcata is distinctly different from the other eight North American alfalfa germplasm sources based on molecular marker analyses (Kidwell et al., 1994; additional marker analyses between 1994 and 2006 will be in a separate report). WISFAL, a tetraploid *M. falcata* population released in 1993, is a syn-2 of 4*x* WISFAL-1 (Bingham, 1990; Bingham, 1993). Sexual polyploidization was used to raise diploid *M. falcata* (2n=2*x*=16) to the cultivated level (2n=4*x*=32). Five generations of 2*x*-4*x* backcrosses with diploid *M. falcata* as the recurrent parent resulted in 4*x* WISFAL-1 which theoretically contains less than 2% *M. sativa* germplasm (Bingham, 1990). These interploiody backcrosses were possible because most diploid *M. falcata* plants produce a low frequency of 2n gametes (Bingham, 1968).

This study compared the morphology, forage quality and productivity of 4*x* WISFAL-1 with its progenitor diploid *M. falcata* population, and two near-isogenic *M. sativa* populations (W2xiso-1 and W4xiso-1), with Vernal.

MATERIALS AND METHODS

Two genetically comparable diploid and tetraploid *M. falcata* and *M. sativa* populations were compared with Vernal. The diploid *M. falcata* population, 2*x* WISFAL-1, is a broad-based synthetic derived from 17 *M. falcata* P.I's. 2*x* WISFAL-1 was used as the recurrent parent to develop the comparative tetraploid, 4*x* WISFAL-1, population (Bingham, 1975; Bingham, 1990). W2xiso-1 and W4xiso-1 are near-isogenic *M. sativa* populations released in 1983 and registered in 1991 (Bingham, 1991). W2xiso-1 is a double-double-cross between 8 CADL genotypes, while W4xiso-1 is its near-isogenic tetraploid equivalent. Both populations have been widely used in physiology and molecular biology studies (Pfeiffer et al., 1980; Volenec, 1988; Kidambi et al., 1991; Brummer et al., 1993). The tetraploid *M. sativa* cultivar Vernal was used as the control. Vernal includes material derived from 2*x*-4*x* crosses with *M. falcata* (Ledingham, 1940) and is particularly winter-hardy and resistant to bacterial wilt.

Plots were hand-sown at a rate of 100 seeds per m, into cultivated soil, at Arlington, Wisconsin on May 20, 1989. Individual plots consisted of three 1-m rows, with 0.5-m between rows and between plots. The experimental design was a randomized complete block design with 5 replicates. The trial site was oversown with *Poa pratensis* L. in August, 1989.

In the establishment year, days to 50% flower, fall regrowth height (mm) and a single forage yield harvest (August 30) were recorded. Fall regrowth height was measured on 12 October to match standard fall dormancy measurements (Sheaffer et al., 1992). During the first production year (1990), plant height (mm) at first flower and three forage yield harvests (June, July and September) were recorded. In the second production year (1991), diploid populations were harvested twice (June and September) and tetraploids three times (June, July, September). Plots were cut as they reached physiological maturity (approx. 10% flower), except for the third cut in 1990 when the diploid populations were harvested prior to flowering. Dryweights were determined on all harvests and data were analyzed using the ANOVA procedure (SAS Institute, 1988).

RESULTS

Morphological differences were detected between ploidy levels of both species. 4*x* WISFAL-1 was 90 mm taller at first flower and reached 50% flower one week earlier than 2*x* WISFAL-1 (Table 1). The 35% increase in plant height of tetraploid *M. sativa* W4xiso-1 relative to the diploid *M. sativa* (W2xiso-1) was greater than that observed for *M. falcata*, while W4xiso-1 also flowered marginally before its near-isogenic diploid (Table 1). Leaves and flowers of 4*x* WISFAL-1 and W4xiso-1 were larger than their diploid counterparts, as expected with higher ploidy. 4*x* WISFAL-1 leaves were smaller and narrower than W4xiso-1 and Vernal.

Regrowth, used here as an indication of fall dormancy, was more vigorous in tetraploid *M. sativa* than diploid *M. sativa* but was similar in diploid and tetraploid *M. falcata* (Table 1). The increased regrowth of W4xiso-1 and 4x WISFAL-1, however, was insufficient to change the fall dormancy classification. Vernal was the least dormant population in this study (Table 1).

Forage Yield

The diploid *M. falcata* established strongly and out-yielded both 4*x* WISFAL-1 and Vernal in the establishment year (Table 1). 4*x* WISFAL-1 also had an establishment year yield greater than Vernal. W4xiso-1, produced significantly more DM than its isogenic W2xiso-1 but less forage than diploid *M. falcata* (Table 1).

Overall, 4*x* WISFAL-1 forage yield was not significantly lower than Vernal (Table 1). 4*x* WISFAL-1 produced nearly 30% more DM than 2*x* WISFAL-1. The main improvement in 4*x* WISFAL-1 was its faster regrowth. W4xiso-1 was better suited to a three-cut system and produced more than double the yield of its isogenic diploid, W2xiso-1, which was the lowest yielding population in this study (Table 1). The diploid *M. sativa* (W2xiso-1) never fully recovered from the three-cut regime in 1990, yielding substantially less DM than all other populations in 1991 (Table 1).

DISCUSSION

Elevating the ploidy level of 4*x* WISFAL-1 gave a marked forage yield increase over diploid *M. falcata*. Leaf size, flower size and plant height also increased in tetraploid *M. falcata*. These morphological changes are consistent with those reported for elevated ploidy level in *M. sativa* (Dunbier et al., 1975; Arbi et al., 1978 and 1979; Pfeiffer et al., 1980; Volenec 1988).

Chromosome-doubling had an even greater impact on the comparative *M. sativa* populations than for *M. falcata*. The two-fold yield advantage of W4xiso-1 over W2xiso-1 confirms previous results from greenhouse and growth cabinet experiments (Pfeiffer et al., 1980; Volenec, 1988). The poor adaptation of diploid *M. sativa* may suggest that not all genes or linkage blocks that are present at the tetraploid level were transfered to the diploid level via haploidy, resulting in a narrower genetic base.

The diploid *M. falcata*'s performed outstandingly in the establishment year. Their yield advantage would have been reduced but not eliminated, had a second cut been taken in 1989, as the regrowth height of the tetraploid *M. sativa*'s was about four times greater than for the diploid *M. falcata*'s. The strong establishment of the diploid *M. falcata* is probably an adaptation to the wild ecosystems in which they evolved. During establishment diploid *M. falcata* appears to allocate resources preferentially into shoot DM in preference to storing carbohydrates in the roots. This is reflected by slower regrowth following first cut in multi-cut systems (McKenzie et al., 1988) and probably adversely affects their long-term yield and persistence.

While tetraploid WISFAL yields are lower than newer cultivars (Holland and Bingham, 1995), it has unique possibilities for alfalfa improvement. Its ability of to cross directly with cultivated alfalfa makes unlimited gene transfer from diploid *M. falcata* possible. Previously 2n gametes were used to transfer genes from diploid *M. falcata* to tetraploid *M. sativa*; now individual plant introductions or improved diploid *M. falcata* populations can be elevated to the tetraploid level using 4*x* WISFAL and crossed directly to *M. sativa* to transfer desirable genes.

Considerable potential exists to improve *M. falcata* at the tetraploid level as little selection pressure has been applied to *M. falcata* compared to *M. sativa*. The hybrid vigor of crosses between *M. falcata* and *M. sativa* has long been recognised (Waldron, 1920; Tysdal et al., 1942; Sriwatanapongse and Wilsie, 1968), and population improvement of tetraploid *M. falcata*'s such as 4*x* WISFAL-1 could improve heterosis with *M. sativa*.

M. falcata reportedly has poorer forage quality than other alfalfa germplasm sources (Lenssen et al., 1991); however, Broderick and Buxton (1991) reported similar ADF or total nitrogen levels in *M. sativa* and *M. falcata* populations, and significantly slower protein degradation rates and higher net protein escape from the rumen in *M. falcata*. Hence, *M. falcata* may have an important future role in improving forage quality and animal performance. Based on preliminary data the forage quality of 4*x* WISFAL-1 seems slightly better than its diploid progenitor.

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Table 1: Morphology, forage quality and forage yield of diploid and tetraploid *Medicago sativa* and *M. falcata*.

Population	Ploidy	Plant height	Days to 50% flower	Fall Regrowth	Crude Protein	ADF	NDF	Forage DM Yield			
								Year 1	Year 2	Year 3[†]	Total
M. falcata		mm	d	mm		g kg^{-1}			g DM m^{-2}		
2x WISFAL	2x	430	93	90	155	327	405	1187	999	927	3114
4x WISFAL	4x	520	86	100	180	319	381	917	1714	1411	4041
M. sativa											
W2xiso-1	2x	370	88	100	172	317	390	735	707	377	1818
W4xiso-1	4x	500	86	190	185	336	395	981	1918	1368	4267
Vernal	4x	550	88	290	171	350	423	764	2175	1516	4455
LSD $_{0.05}$		18	1.5	12	12	23	22	120	266	297	547
CV (%)		5	2.1	12	5.3	5.2	4.2	10	14	20	12

[†] diploids harvested on two-cut regime in third year

[2] crude protein, ADF and NDF adjusted using mean stage by weight as a covariate.