SYNOPSIS OF ALFALFA POLYPLOID RESEARCH

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Cultivated alfalfa was recognized as a tetraploid (2n = 32 chromosomes) early in the century. Uncertainty about allotetraploidy versus autotetraploidy persisted until 1952 when Stanford published his benchmark paper on tetrasomic inheritance (18). Since then alfalfa has been considered an autotetraploid (2n=4x=32), and chromosome pairing in haploids reinforced the conclusion (19).

Two keys to making ploidy level comparisons in alfalfa were i) the production of CADL to make cultivated alfalfa germplasm available as diploid germplasm (4) and ii) the development of isogenic populations at multiple ploidy levels (3, 9, 10, 15, 22). These two steps were involved in most of the subsequent agronomic, physiological and breeding comparisons. Ploidy research used sets of genetically related and/or isogenic populations at 2x - 4x, 4x - 6x, and 4x - 8x levels. Use of populations avoided the problem of inbreeding that results from chromosome doubling. Another problem was that the harvest schedule for standard 4x alfalfa usually was not suitable for the other ploidy levels. A field plan that permitted separate harvest dates was useful. Tetraploid alfalfa has greater seedling growth rates than diploid alfalfa due to larger seed size. While 4x out yields 2x, the herbage relative growth rates are similar. Crowns of 4x are larger than 2x and larger shoots on crowns after harvest contribute to greater herbage regrowth. Tetraploids have greater shoot length with significantly more leaves per shoot and twice as much area per leaf but similar numbers of shoots per plant compared to diploids (20).

In general, cell and organ size increases with an increase in ploidy level. Stomatal size and number of chloroplasts per guard cell increase with ploidy level and can be used in screening (1). Stomatal densities in terms of the number of stomates in a given unit of area always decrease with an increase in ploidy (16). Concentrations of protein and nitrogen were similar in 2x and 4x, but dry matter, leaf weight, and glucose-6 phosphate dehydrogenase activity per leaf were about double in the 4x (9). On a single leaf basis, carbon exchange rates were similar in 2x, 4x, and 8x. As the ploidy level doubles from 2x - 4x the fresh weight per cell doubles. Amount of DNA, RuBP carboxylase, chlorophyll, coupling factor 1, number of chloroplasts, and photosynthesis per cell also double. Because cell size doubles but cells per leaf area decrease, photosynthetic rate per leaf area does not change. As the ploidy level doubles from 4x - 8x the fresh weight per cell increases only 50%. Chlorophyll and number of chloroplasts per cell double while DNA, RuBP carboxylase, coupling factor 1 and photosynthesis per cell only increase 50 - 67% (21). Net carbon dioxide exchange on a whole plant basis was equivalent across ploidy levels, as was acetylene reduction and nodule weight (15). Additional references to physiological and stress parameters of 2x and 4x may be found in a review (5).

Overall, ploidy per se has very little effect on physiological processes expressed on a per leaf or per plant basis. The 4x level exceeds the 2x level in plant size, and while the 6x level could potentially be beneficial due to the increased size associated with polyploidy, it is reproductively unstable (2, 17). The 8x apparently exceeds a ploidy optimum as it is sensitive to stress, particularly water stress, and it is unstable somatically and reproductively (3). The 4x level will remain the cultivated form of alfalfa.

Differences in diploid and tetraploid combining ability were noted in 1988 (10). Research on genetic diversity as measured by differences in lengths of homologous DNA restriction fragments found that herbage yield in tetraploids was more responsive to genetic diversity than in diploids (13). A genetic model to explain these results was developed (5) based on previous research on progress heterosis in tetraploids (7, 8, 11) and genetic improvements in two allele populations (14, 22). The model explains all aspects of 2x and 4x breeding behavior studied over the years. The key features are favourable dominant alleles at different loci and linkage blocks (Fig. 1). The favourable dominants potentially complement each other, and the more dominants the better. Regarding 2x versus 4x differences, note that any two different linkage blocks possessed by a diploid would contain fewer dominants and less potential complementary gene action than four different blocks in the 4x. The differences between 2x and 4x alfalfa in combining ability, responsiveness to DNA diversity, as well as vigour and yield, all can be explained by the model.

The severe inbreeding depression generally observed in alfalfa (6, 12) also can be explained by the model. The state where there is a dominant at each locus drops from 100% to 16.6% with one generation of selfing. This loss of complementary gene action and interaction can easily explain the severe inbreeding depression in alfalfa. This notion was first attributed to loss of first order interactions from multiple allelic loci (6) which are now considered to be the linkage blocks i, j, k, l, in Fig. 1 (5). In conclusion, most ploidy relations and the breeding behaviour of alfalfa can be explained by mechanisms that are probably common to other autopolyploid species. Tetraploid alfalfa best combines agronomic performance with reproductive stability.

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i	j	k	l
A	a	a	a
b	B	b	b
c	c	C	c
d	d	d	D

Figure 1. Model of a tetrasomic chromosome position containing four different linkage blocks i, j, k, and l. In the model each block contains one favourable allele.