**GENETICS OF RED ROOT ALFALFA: A useful marker in spite of segregation distortion**

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Introduction

Red root alfalfa was mentioned by Tysdal and Westover in the 1937 Yearbook of Agriculture. They indicated that R. A. Brink at the University of Wisconsin was working with a dominant red root marker. The material was tetraploid. Hence, Brink had it in his program, but never published on it. The remnant red root stock from his program was labeled >Ladak=. D. K. Barnes (MN) and I also worked with it over our careers, and many times discussed the fact that we could not stabilize it in the homozygous condition. By the 1980's we quit trying to obtain the stable homozygous condition and started to use presumed triplex genotypes for certain genetic marker purposes. They were essentially true breeding. Three such presumed triplex genotypes tested as seed parents produced 1 normal in ca 300 progeny. We assumed that the normals were due to either double reduction or numerical non-disjunction.

Barnes and Hanson 1967 in Tech. Bull. 1370 indicated that E. H. Stanford found transmission problems on the male-side (pollen transmission problems). We had seen second generation segregation that indicated even the best red root parents were not homozygous. Hence we thought the male gamete had to be heterozygous. This could be due to a lethal for male gametophyte development or for pollen function. Hence, we made a detailed study of male and female transmission. The upshot of the following is that our best red root parents may be homozygous, but that there are transmission problems with one or more chromosome blocks carrying the red root gene, or transmission problems with the red root gene per se. Fortunately, there is at least one red root gene in the pollen even when there is a transmission problem, which ensures the usefulness of the marker. On occasion there is a female transmission problem, perhaps when the female is duplex for one or two copies of the affected gene. And, male transmission problems are more frequent than female problems. Both female and male transmission problems result in segregation distortion. The results suggest a new round of experiments. We are well aware of imprinting and will keep this possibility in mind in the future research.

The main reason that we are continuing to study red root is because it is such a useful seedling marker. Also, two verified haploids literally fell out of the best all around clone on our project (>Blazer XL=-Clone P), in a cross where an extremely good red root parent (clone E1) was the pollen parent. The haploids were found along with hybrids and self progeny of clone P after crossing clone P with pollen from clone E1. It is like manna from heaven to get free haploids of a favorite clone. And, it is a clear message to learn as much as possible about the transmission of the red root trait. We realize that the haploids may be spontaneous, but this doesn=t seem likely.

Red Root Chronology

1937 Mentioned by Tysdal and Westover in The Yearbook of Agriculture.

1967 Red root is mentioned as a genetic trait by Barnes and Hanson.

1968 Bingham found the Wisconsin red root source while digging alfalfa plants at Marshfield, WI. Then, he traced it back to a remnant seed envelope descending from R. A. Brink=s project and the envelop indicated >Ladak= origin. About this same time, Bingham found some plants that had red-pigmented bands on the root. He never could recover the phenotype in self progeny and dropped the study. Some old 35 mm slides remain. Could the bands, which were spaced irregularly have been due to a mutable red root allele?

1968-1990 Red root inheritance of the Wisconsin (Ladak) source was studied almost every year. It was transferred into various backgrounds. It was used in several studies perhaps the biggest one was to study pollen movement as it relates to containment of transformed traits in biotechnology. At Arlington, WI, we were able to find the trait as far away as one mile from the source. We concluded that containment could not be guaranteed, especially considering wind-blown or hitch-hiking bees.

1990 A red root seed increase was made at Prosser, WA as part of NC-83 project resulting in seed stock WI 90-10. Intercross seed of a number of northern - adapted, Vernal, and proprietary breeding stocks that were Anearly true breeding@ was sent to Prosser for seed increase.

1994 Red root was used in a Apollen-racing@ study because we had always been concerned about its effect on pollen. Five simplex (Rrrr) plants were used as male parents in crosses to four cultivar-type plants (one male sterile, one cream flowered, and two partial inbreds that were self-sterile). The bottom seed of each pod was harvested and studied to determine the frequency that Rr versus rr gametes fertilized the bottom ovule. The race to the most distant ovule was a tie. The ratio of red root to normal progeny fitted a 1:1 ratio. Thus, red root in the heterozygous condition in the pollen of tetraploids was competitive with normal pollen. What about when it is in the homozygous condition? Clone E1 has been used in many studies and much of its pollen is homozygous for two copies of red root. Pollen of E1 is competitive in crosses as indicated by crossing percentages. The crossing percentage of E1 as a pollen parent on typical cultivar plants averages about 90% in the greenhouse with a range of 80-100%. It was learned in these studies that some cultivar plants that are relatively self-fertile in the absence of cross pollen produce almost no self seed in crosses. There has been good agreement between E1 and WISFAL on crossing percentage. We had assumed that highly self-fertile plants all contributed to the self-seed content in cultivar seed production, but this does not appear to be the case. There is a

wide range in pollen competition, and Asuper@ pollen parents probably are predominating in many crossing strategies. Red root is very useful in studying pollen competition and likely will be used in future research. We find it useful in spite of the segregation distortion because the pollen of the tetraploid almost always carries at least one dominant red-root gene.
1996 A plant in the WI90-10 population was selected as possessing the best combination extreme red root, vigor and fertility of ca 1000 plants examined. It was code named E1, and E1 is used in this write-up for convenience.

1998 E1 was essentially true breeding in crosses, i.e. almost all progeny (at least 299 of 300) were red rooted when E1 was crossed as a female or male with normal. From a cross of E1 x Columbia 2000 (C2K) and its reciprocal C2K x E1, we crossed 25 F1 red root plants from each combination onto male sterile 6-4 ms. 6-4 ms x (E1 x C2K) tested female transmission in the first cross of the chromosomes carrying the red root genes. 6-4 ms x (C2K x E1) tested male transmission.

The genetic situations in our experiments are as follows:

Genetic Models

1) Assuming E1 is homozygous (RRRR), the F1 plants would all be duplex (RRrr). They would segregate 5 red root to l normal in the test cross.

6-4 ms X (E1 x C2K)
(rrrr) (RRrr)
Gametes 1RR 4Rr 1rr
and progeny RRrr Rrrr rrrr
All families segregate 5 red root-to 1 normal

2) Assuming E1 is triplex (RRRr)

6-4 ms X (E1 x C2K)
(rrrrr) 2 RRrr + 2 Rrrr
Gametes 1RR 4Rr 1rr + 1Rr 1rr
and progeny RRrr Rrrr rrrr Rrrr rrrr
One-half of families segregate 5:1, and
One-half of families segregate 1:1

2000 A test of the genetic models was completed. In the test, if E1 is homozygous, then all F1s would be duplex and all test cross families would segregate 5 red root to 1 normal. In the test of female transmission, 17 of 20 test cross families segregated 5:1. This indicates that E1 is homozygous, but there is a transmission problem. The three other families segregated about 2:1, about 1:1, and about 1:5, respectively. These are aberrant transmission ratios. They also qualify as some form of segregation distortion. The transmission problem is more frequent on the male side. Only 8 of 15 test cross families segregated 5:1,

and one would conclude from this that E1 could not be homozygous. The other families segregated about 2:1 (4 families), and about 1:1 (3 families). Interestingly, there was no family on the male side that was as aberrant as the family on the female transmission side that segregated 1 red root to 5 normal.

## Discussion

Rather than speculate about the reasons for the segregation distortions, we plan to do some additional experiments. The plan is to establish simplex pedigreed lines while backcrossing into elite cultivar backgrounds, and at some point resyntheses quadruplex homozygotes of individual blocks/alleles. We have not individualized the blocks/alleles previously and this may be all that is needed to obtain a stable homozygous red root stock. Also, we will try to haploidize tetraploid red root plants to transfer red root to the diploid level where the genetic studies are easier. We will try to scale down red root even though for some reason we could not do it 20 years ago. We could not obtain red root haploids, and, although we had two or three red root triploids, they did not transmit red root to progeny in 3x-2x crosses. Maybe we were just unlucky, or maybe we lost interest too quickly. Hence, we will try again.

## Epilogue (January 2001)

Backcrossing has begun to individualize the chromosome blocks with respective red root alleles in clone E1. In addition to trying to develop a stable red root stock, we will try to identify the factor that may be promoting haploidy. It could be associated with the transmission problem and SD. There is reason to believe that the transmission problems are in the gametes of E1, in which case they should show up in self progeny of E1. Another array of plants from WI90-10 also will be tested. Do all the red root plants behave like E1? Are there plants even more interesting than E1?

There is one more possible explanation for the difference in male and female transmissions that I can think of: that E1 is triplex as indicated by the male data, but that the red root gene is preferentially transmitted on the female side because of the linear formation of the female gametophyte and some chromosome phenomenon such as a translocation. An analysis of meiosis in pollen mother cells should be done. There may be even more things to look for in the future work.