

FREQUENCY AND FUNCTION OF 2N GAMETES IN ALFALFA CULTIVARS

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This is a progress report of many years of research. In 1992, Kidwell found two octoploid S1 progeny of a plant from W-10 AC3 germplasm (2). Reserve S1 seed produced 20 tetraploid and another octoploid plant. The 20 tetraploids were crossed as seed parents with several conventional cultivar pollen parents and produced from 1 - 20% octoploids among tetraploid in about 100 F1 progeny of each. None of cultivar pollen parents appeared to produce 2n pollen in a cursory search and we suspected that there was an embryonic chromosome doubling mechanism.

Since 1992 the genetic tendency to produce octoploids has been backcrossed into standard cultivars including inbreds of '>Columbia 2000'. In 1998, the new genetic backgrounds produced hexaploids as well as octoploids. This meant that 2n gametes not an embryonic doubling was involved. The explanation for finding only octoploids earlier is that there is a strong 6x block in seed development in some stocks, and that conventional cultivars have more 2n pollen than we realized. Back in 1968, two hexaploids were discovered in Saranac (1) and we are examining Saranac again to determine if it has a weaker 6x block than most cultivars.

In 1999, samples of WISFAL, Vernal, Legendairy and 10 other cultivars were screened for occurrence of 2n pollen in about 200 - 400 pollen grains of each examined under the microscope. The screen was very conservative and a higher frequency of cultivar plants producing 2n pollen likely would be found in larger samples.

Plants producing 2n pollen were found in every cultivar where at least 60 plants were examined. The frequency of plants producing 2n pollen was 1-5% of the cultivar plants. Ongoing research indicates that at least one Legendairy plant is producing both 2n eggs and pollen and about half of its self progeny are in the hexaploid - octoploid range. The basic mechanism of 2n pollen formation in cultivars appears to be disoriented spindle at Anaphase II as was previously reported in diploid alfalfa (3).

Since the evolution and breeding of cultivated alfalfa is literally based on 2n gametes in diploid progenitors (e.g. Vernal and Narragansett, and very likely Grimm, Cossack, and others) it is not surprising that 2n gametes also occur at the tetraploid level. In our experience, fertility of 2n gamete producers is only slightly less than normal, and there probably is only mild selection against them. The occurrence of spontaneous sexual polyploids at hexaploid and octoploid levels is very low and likely does not affect cultivar performance. Knowledge about 2n gametes contributes to our understanding of the reproductive biology of alfalfa. Our long range goal is to select against 2n gametes in regular breeding

material, while also studying them for basic knowledge and novel breeding methods.

References

1. Bingham, E. T., Binek, A. (1969) Hexaploid alfalfa, *Medicago sativa* L.: Origin, fertility and cytology. *Can. J. Genet. Cytol.* 11:359-366.
2. Elgin, J. H. (1982) Registration of W10 multiple pest resistant alfalfa germplasm. *Crop Science* 22 (1):163.
3. Vorsa, N., Bingham, E. T. (1979) Cytology of 2n formation in diploid alfalfa, *Medicago sativa*. *Can. J. Genet. Cytol.* 21:525-530.