Proceedings of the Iowa Academy of Science

Volume 70 | Annual Issue Article 30

1963

A Preliminary Study of Chromosome Number in Oak Sprouts

David F. Conover
Wisconsin State College

Literature Cited

1. Godward, M. B. E. 1948. Nature (Lond) 161:203.
2. Venkataraman, G. S., and K. V. Natarajan. 1959. Stain Technol. 34: 233-234.
3. Wittman, W. 1962. Stain Technol. 37:27-30.
4. Beeks, R. M. 1955. El Aliso 3:131-134.
5. Alexopoulos, C. J., and E. S. Beneke. 1962. Laboratory Manual for Introductory Mycology. Burgess, Minneapolis.
6. Starr, R. C. 1960. Am. J. Botany 47:67-86.
7. Anderson, L. E. 1954. Bryologist 57:242-244.

A Preliminary Study of Chromosome Number in Oak Sprouts[1]

DAVID F. CONOVER[2]

Abstract. A preliminary study of chromosome numbers in oak sprouts was undertaken to determine the frequency of polyploid stump sprouts. A satisfactory method of making slide mounts using lateral bud primordia of newly burst buds was developed. None of the limited number examined to date was polyploid.

For some time it has been known that sprouts developing on excised stems are occasionally polyploid. Jorgensen (1928) was able to obtain up to 10 per cent tetraploid tomatoes by decapitating young tomato plants and removing all buds. A callus tissue developed on the wound surface producing numerous shoots, most of which were diploid. Lindstrom and Koos (1931) using homozygous diploids (from a sprouting haploid) obtained about 32 per cent tetraploid sprouts. The possibility that some of the peculiarities observed in oak stump sprouts might be attributed to polyploidy was the incentive for a study of chromosome numbers in oak stump sprouts.

MATERIALS AND METHODS

A preliminary study, primarily to work out a technique of determining chromosome number of sprouts, was initiated in late December 1961. Twenty-six pin oak (*Quercus palustris* Muenchh.) sprouts were collected and placed in the greenhouse on December 27. All 26 were taken from two small 4 in. diameter stumps in the State Conservation Commission nursery south of Ames. Additional sprouts were collected March 12, 1962. Each sprout was placed either in tap water or in moist sand within a plastic terrarium. As soon as the buds began to break dormancy and had elongated ½-1 in., collections were made. Buds were placed

[1] Journal Paper No. J-4335 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1047. In cooperation with the United States Department of Agriculture. Sincere appreciation is due Dr. H. S. McNabb, Jr. for encouragement during the course of this study.
[2] Department of Biology, Wisconsin State College, Oshkosh, Wisconsin.

in 0.2 per cent Actidione R in tap water for four hours to shrink the chromosomes and maintain them in prophase. The young stem tips were then fixed in three parts absolute alcohol to one part glacial acetic acid for 15 minutes at 60°C. Hydrolysis was generally 10 minutes in 1N HCl followed by staining using the Feulgen nucleal reaction.

Work with oak chromosomes cited in the past has been with root tips or with anther squash techniques. Some difficulty was experienced with emerging buds because the apical meristem was surrounded by large numbers of bracts (two at the base of each leaf) and leaves. The leaves became mushy under hydrolysis, making it difficult to find and remove the dividing area at the apex. This problem was resolved by using the lateral bud primordia which develop very rapidly as the new stem elongates. The best material was obtained when the leaves were well developed but still appressed to the stem axis. Primordia were separated from the stem with fine needles, and each provided a small quantity of dividing tissue. Cover slips were placed over the mounds of tissue and tapped to spread and flatten the cells as much as possible. Although single cells were difficult to isolate by this technique, counts could be made from surface cells. This was adequate to determine whether or not the sprouts were polyploid. The tissue was quick-frozen using liquid CO_2 (Bowen, 1953), dehydrated in 95 per cent alcohol, containing fast green as a counterstain and mounted in Euparal.

Results

All sprouts collected in December broke dormancy in 4 to 6 weeks. Sprouts collected in March broke dormancy in 2½ to 4 weeks. In general, larger sprouts exhibited more vigorous growth and facilitated production of acceptable material. Of the 40 sprouts examined, all were diploid as determined by counts of prophase chromosomes (32 cases) or by nuclear measurements (8 cases). The sprouts were not selected for any unusual characteristics. Rather, a 100 per cent sample was taken which included sprouts from the root collar region as well as from the stump sides and tops.

An effort was made to photograph the chromosomes of oak, since to my knowledge, only drawings have previously been published (Figure 1). Furthermore, considerable controversy existed at one time as to whether oak in the normal diploid state had 22 (Wetzel, 1929), 12 (Friesner, 1929) or 24 (Høeg, 1925) chromosomes. This confusion undoubtedly resulted from attempts made to count chromosomes from paraffin sections of somatic tissues (usually roots). In more recent work, the diploid chromosome number of 24 has been confirmed for all native

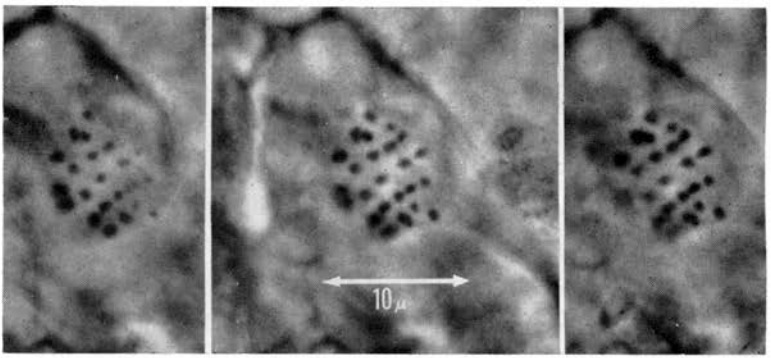

Figure 1. Oak chromosomes of prophase, treated with Actidione R and stained by the Feulgen method: Center photograph shows 24 chromosomes; left and right flanking photographs improve definition of periferal chromosomes by altering plane of focus; phase contrast. (Courtesy of L. Facto)

species of oaks thus far investigated (Darlington and Wylie, 1956).

Discussion

The sample taken thus far is not large enough to indicate presence or absence of polyploid oak stump sprouts. The percentages obtained by workers with tomato were high because all sprouts developed from callus tissue. In the case of oak stumps, the majority of sprouts which occur are commonly thought to be from bud traces which proliferate from the original lateral buds of the seedling and grow enough to keep up with the diameter growth each year. This assumption will be critically investigated. A few sprouts arise from callus tissue at the top surface of the cut stump, and might be expected to produce occasional polyploid sprouts. Collections will be continued on natural populations of sprouts to determine what, if any, correlation exists between irregularities found in oak sprouts and chromosome number.

Literature Cited

Bowen, C. C. 1956. Freezing by liquid carbon dioxide in making slides permanent. Stain Techn., 28:281-283.

Darlington, C. D. and A Wylie. 1956. Chromosomes atlas of flowering plants. 519 p. Macmillan Co., New York.

Friesner, R. C. 1929. Chromosome numbers in ten species of Quercus with some remarks on the contributions of cytology to taxonomy. Bot. Stud. Butler Univ. 1:77-103.

Høeg, Eiler. 1925. Om mellem former ne mellem *Quercus robur* L. og *Q. sessiliflora* Martyn. Botanisk Tidsskrift. 40:411-427.

Jorgensen, C. A. 1928. The experimental formation of heteroploid plants in the genus Solanum. Jour. Genetics 19:135-211.

Lindstrom, E. W. and K. Koos. 1931. Cytogenetic investigations of a haploid tomato and its diploid and tetraploid progeny. Am. Jour. Bot. 18: 398-409.

Wetzel, Gerhard. 1929. Chromosome studien bei den Fagales. Bot. Archiv. 25:258-283.