



OREGON STATE UNIVERSITY SEED LABORATORY

PLOIDY BY FLOW CYTOMETRY

AN IDEAL TECHNOLOGY FOR DETERMINING THE PLOIDY LEVEL IN RYEGRASS AND OTHER SPECIES

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In the modern seed industry systems, seed testing methods are useful for: 1) generating critical information to manage seed quality efficiently during the development production processes, and 2) labeling of finished seed products correctly as they are prepared for sale. The ploidy test by flow cytometer, offered by Oregon State University Seed Laboratory, is used for the above two applications to identify the ploidy levels of various crops.

A decade ago, determining the ploidy level of ryegrass samples was not an easy task. It relied in counting chromosomes on squashed root-tips, which was extremely time consuming and subjective. Needless to say that this method placed serious limitations to the sample size and the number of cells in each root that could be evaluated, in addition, seedlings had to be evaluated in a specific age. The ploidy by cytometry, on the other hand is objective, accurate, and can be used to test plant tissues from seedlings, plants, or buds. It also can be used to screen hundreds or thousands of plants to determine their ploidy level. Thus, ploidy by cytometry has replaced the chromosome counting technique.

The need for a test to distinguish between diploid and tetraploid ryegrass at the laboratory has emerged as a result of the increased use of tetraploid cultivars and the interest of seed industry to differentiate between tetraploid and diploid seed lots in the market channels. The Oregon State University Seed Laboratory responded by introducing the ploidy by cytometry method in 1998.

THE PRINCIPLE OF TEST

The ploidy level (e.g., diploid, tetraploid, etc.) in a sample is determined by measuring the amount of nuclear DNA in plant cells. The *flow cytometer* automatically, rapidly and accurately makes this determination. Figure 1 shows a peak in channel 100 (in the x-axis) indicating that the sample is pure tetraploid ryegrass. In contrast, figure 2.

shows a peak in channel 100 (tetraploid) and another peak in channel 50 (diploid) indicating that the sample has a mixture of diploid and tetraploid ryegrass.

Among the advantages of this method are: the objectivity in interpreting the results; the records of the test results are maintained in the flow cytometer for future reference; the test does not damage the sample; the test is repeatable; and finally plant tissues from germinating seedlings, greenhouse plants or from field stands can be used. This allows customers to have the information they need and keep the seedlings or the plants used in the test intact. This can be useful for various applications.

WHO CAN BENEFIT FROM THIS TEST?

Ploidy by cytometry test provides valuable information regarding the ploidy level of various plant species. It compares the ploidy level of various germplasm collections, test the ploidy levels of parents and crosses, and detect ploidy contamination in seed samples. It can be used by a wide range of users including:

- ***Plant breeders:*** A breeder can send unknown ploidy level seeds and ask to screen and return only seedlings with certain ploidy level, e.g., the tetraploid seedlings; or can send greenhouse or field plants for ploidy identification. Obviously, this is an invaluable preventive practice that can eliminate potential problems that may happen when a breeder seeds with mixed ploidy levels are used to start larger seed increase programs. No wonder, some breeders are doing just that.
- ***Seed growers:*** They can use the test to verify the genetic purity of their seed stocks before planting. Also, to monitor the potential ploidy contamination as a result of volunteer plants from previous crops or off types that show up in the field.
- ***Seed certification inspectors:*** The test can be used by field inspectors to monitor ryegrass fields for off types to determine if they are diploid or tetraploid. The test is also used to complete the certification process and issue the labels for the end products.
- ***Seed dealers/buyers:*** Seed dealers can use the information to label their seeds correctly and to provide ploidy information to their buyers. Seed buyers can use the test to verify the ploidy level of the seeds they purchase.
- ***Seed control officials:*** to verify compliance with truthful in labeling.
- ***Researchers:*** The test can provide useful information to researchers who need information on the ploidy level of germplasm collections, outcrossing (contamination through pollen from neighboring fields), and for other ploidy research objectives. An example is the verification of the ploidy level of various fine fescues that are different in chromosome count, e.g., strong creeping red fescue $2n=56$; slender creeping red

fescue $2n=42$; sheep fescue $2n=28$; hair fescue $2n=14$. Other research examples that have been conducted at the OSU Seed Lab are the determination of ploidy levels of wild and cultivated tomatoes; screening *Brassica* and *Agrostis* species collected from various areas of the world; and compare the ploidy level of various crosses between wheat and jointed goatgrass (*Aegilops spp.*), a weed relative of wheat.

The ploidy by cytometry test was used at the Oregon State University Seed Laboratory first to determine the ploidy level of tetraploid ryegrass varieties as a part of the certification process. Since then, its application has gradually extended to provide valuable services to plant breeders, growers, seed dealers, certification inspectors, and researchers. It is worthy to note that determining the ploidy by cytometry is not restricted to ryegrass, it can be used to determine the ploidy levels of field crops, shrubs, trees, natives, and other species. There is no doubt that ploidy by cytometry is the technology of this century for ploidy determination.

For any question regarding ploidy by cytometry, contact the Oregon State University Seed Laboratory at 541-737-4464 or e-mail seedlab@oscs.oregonstate.edu

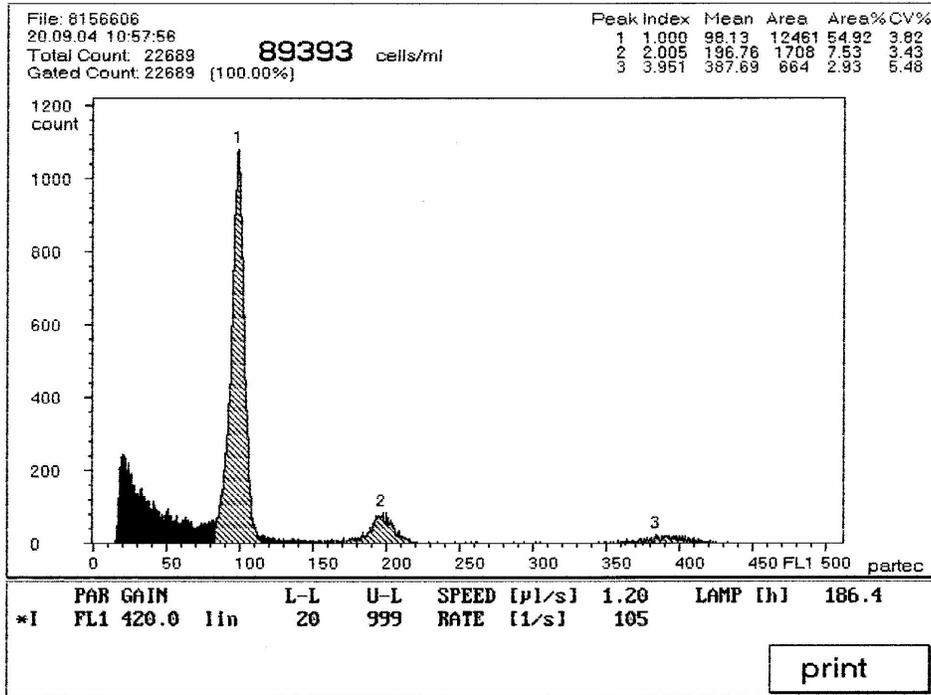


Figure 1. Pure tetraploid ryegrass sample, showing a peak in channel 100, as measured by the flow cytometer.

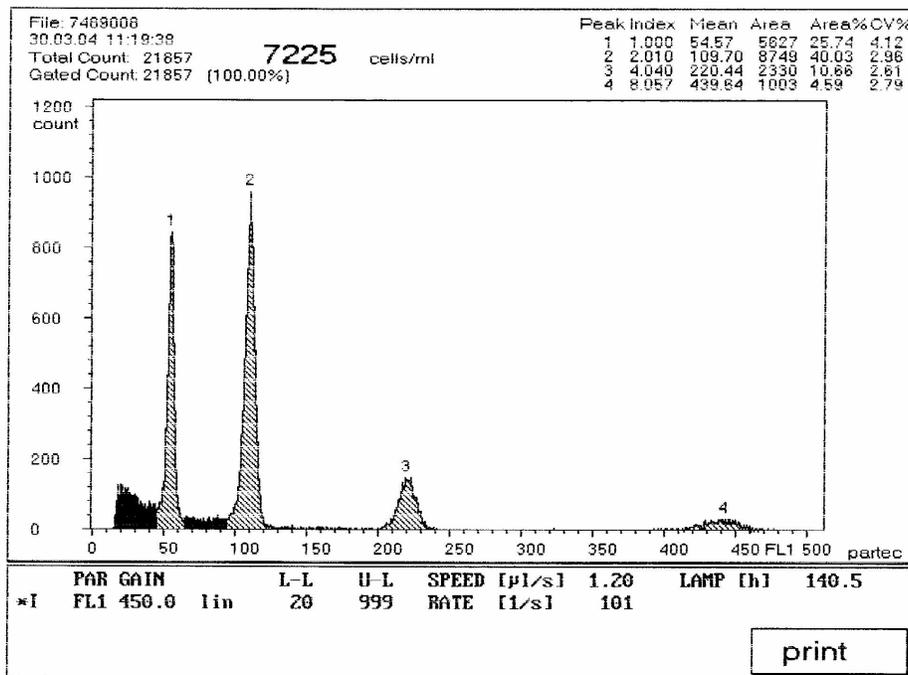


Figure 2. A ryegrass sample showing two peaks, one at 100 and another at 50 channel (on the x-axis) indicating a mixture of diploid and tetraploid as detected by the flow cytometer.