

DNA Markers...

Americans eat nearly 8 pounds of dry beans each year in soups, salads, and other dishes and more than 7 pounds of frozen, canned, or fresh snap beans. But only the best bean cultivars make the cut when it comes to traits that will help growers profit and satisfy consumer tastes.

One cultivar trait farmers prize most is disease resistance, especially against



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Above: Plant geneticists Phil Miklas (left) and George Vandemark inspect damaged snap bean plants at Prosser, Washington.

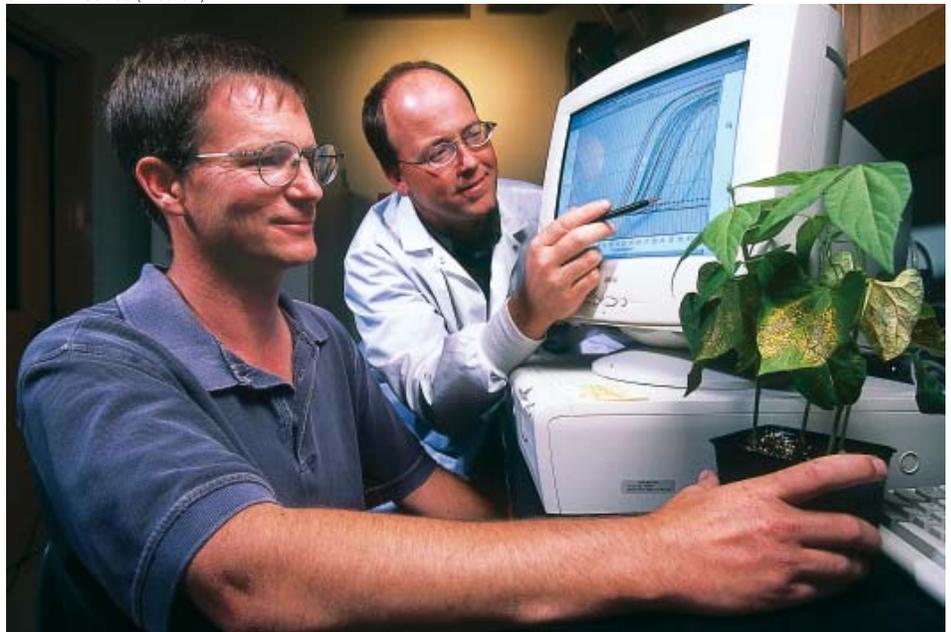
Left: Phil Miklas inoculates selected bean plants with a virus.

With a Twist for Improved Bean Breeding

bean common mosaic virus and bean common mosaic necrosis virus. Severe outbreaks of these aphid-borne viruses can cause yield losses of up to 60 percent and threaten the \$512 million dry bean crop of California, Colorado, Idaho, Michigan, Nebraska, North Dakota, and Washington. Also at risk is the \$190 million snap bean crop of Florida, Illinois, New York, Oregon, Wisconsin, and other states.

Forefront in the war on such viruses is marker-assisted selection (MAS). It's a biotech approach some breeders are using to rapidly identify resistant plants by confirming the presence of certain genes rather than by observing actual disease symptoms induced in a greenhouse. Now, an advance by ARS scientists in Prosser, Washington, could make MAS even more useful by enabling it to do what it often couldn't before: distinguish

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Phil Miklas and George Vandemark analyze results of quantitative polymerase chain reaction assays used to rapidly genotype bean plants for a virus-resistance gene.



homozygous from heterozygous plants. Homozygous plants carry the desirable gene on each of the two chromosomes. Heterozygous plants carry the beneficial gene on one chromosome, while the other chromosome contains a slightly different version of the gene that does not confer the desirable trait.

The advance, called co-dominant interpretation, should save breeders

substantial time, labor, and money spent ensuring that promising cultivars are homozygous, or “true breeding,” meaning their offspring will consistently show the same traits over many generations.

In beans, the gene for resistance to the two viruses is dominant, say George Vandemark and Phillip Miklas, plant geneticists at ARS’ Vegetable and Forage Crops Production Research Unit. When a gene is dominant, a single copy in a heterozygous plant will confer the desirable trait, making the plant visually indistinguishable from the true-breeding, homozygous plant. This presents an obvious problem in excluding heterozygous individuals from the pool of plants used for seed generation, say the scientists.

X Marks the Spot

In some quarters, classical plant breeding techniques have given way to MAS because it detects certain traits more easily. The markers themselves are specific sequences of DNA inside the plant’s chromosomes. A marker serves as a road sign that says a desired gene is close by.

Key to finding markers is the polymerase chain reaction (PCR). It’s the

same technology that forensics experts use to identify DNA fingerprints in blood, hair, or other biological samples. But bean breeders use it to search for plants that possess a specific virus-resistance gene called *bc-1²*. This gene, which confers resistance to most bean mosaic virus strains, is often bred into pinto, navy, and other dry beans as well as snap beans.

A breeder prefers plants with two identical copies (homozygous) of the virus-resistance gene because the plants will be true breeding, meaning all their offspring will be virus resistant. But if the plant is heterozygous, it will have one copy of the *bc-1²* resistance gene and one copy of an alternate gene called *bc-1*, which confers susceptibility to most bean mosaic virus strains. Heterozygous plants won’t breed true, and some of their offspring will be susceptible to virus attack.

Breeders want to exclude the *bc-1* gene from their breeding programs, but this is difficult with existing PCR detection methods, Miklas says. Breeders now rely on a process called progeny testing, where the plants’ offspring (progeny) are tested for resistance to the virus in a greenhouse. Such testing allows breeders to detect heterozygous plants and eliminate them from the breeding program. This process can take 6 months to 1 year and adds considerably to the cost of breeding programs.

Making a Good Biotech Tool Better

Use of DNA markers typically begins with PCR to identify plants having the *bc-1²* resistance gene. Until recently, the PCR technique was able to detect the *bc-1²* gene but not the number of copies present.

Vandemark and Miklas overcame this problem by using a newer PCR method that can accurately tell how much marker DNA is present by labeling it with a fluorescent compound and then measuring the fluorescence. “Since our quantitative PCR assay is specific for the *bc-1²* gene, we can expect that homozygous plants will fluoresce twice as much as heterozygous plants,” says Vandemark.

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George Vandemark isolates DNA from a bean plant that will be examined with a polymerase chain reaction assay to determine whether it has the virus-resistant gene. The assay can be completed in 1 day, whereas greenhouse methods require up to 4 months to determine the same thing.

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Phil Miklas compares susceptible (left) and resistant (right) bean lines exposed to virus infection.

Snap beans that possess the gene *bc-1²* are resistant to most strains of bean common mosaic virus and bean common mosaic necrosis virus.

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STEPHEN AUSMUS (K10343-1)



Phil Miklas and George Vandemark examine healthy, disease-resistant snap (left) and pinto bean breeding lines developed at Prosser, Washington.

that will help growers protect their crops from viral diseases.

The scientists reported their advance in a recent issue of the journal *Molecular Breeding*.—By **Jan Suszkiw**, ARS.

This research is part of Rangeland, Pasture, and Forages (#205) and Genomic Characterization and Genetic Improvement (#301), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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