BREEDING FOR RESISTANCE TO THE BLACKLINE VIRUS: INCREASING THE SPEED AND EFFICIENCY OF TRADITIONAL BACKCROSSING BY INTEGRATING MOLECULAR TECHNIQUES.

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ABSTRACT

Walnut blackline disease caused by the cherry leafroll virus (CLRV) causes fatal necrosis at the union between English walnut scions and hypersensitive rootstocks. All English walnut cultivars tested so far can be systemically infected and as a result the virus can move through the English walnut tree to the hypersensitive rootstock and cause blackline girdling. A breeding program for developing a blackline resistant English walnut cultivar was begun in 1983. The time required to breed an English walnut cultivar that is resistant to CLRV can be significantly decreased by integrating new molecular techniques with traditional backcrossing. The current blackline resistance breeding stock is a population of about 140 first generation backcrosses (BC1s), 250 BC2 items and their parents. To increase this population, over 1200 controlled crosses were made in 1992. The resulting seeds (330 BC2 seeds and 23 BC3 seeds) will be planted to a nursery in the spring of 1993. Selection among these seedlings will be based on seedling morphology and phenology, as well as on seedling DNA, will permit much more rapid progress than simple backcrossing. Morphological and phenological observations were made on over 400 items in the blackline breeding program and added to a database for analysis. Crackout, yield and nut quality data were taken from a sample of the items in the program. The observed variation was correlated with the percent English walnut genome present in the item. DNA was collected and isolated from about 90 members of the breeding populations. Analysis of the DNA has uncovered 30 DNA polymorphisms that can be used to determine which backcross seedlings are most promising. Seventeen BC2 accessions were identified as hypersensitive to CLRV.

OBJECTIVES

While backcross breeding is a proven and effective way to introduce disease resistance into crops, backcrossing is slow and inefficient in crops such as walnut with long generation times. The objective of this proposal is to increase the efficiency and speed of backcross breeding for resistance to blackline by augmenting traditional backcrossing and selection techniques with new, biochemical methods for evaluating backcross progeny. By using methods which permit direct screening and comparison of the DNA of backcross progeny, potentially outstanding individuals should be more rapidly and accurately identified and brought to maturity than through the use of conventional morphological evaluations alone.

Work done in 1990-1992 is part of a long-term backcross breeding program with the goal of developing and releasing a blackline resistant English walnut cultivar.
PROCEDURE

A population of about 80 (black X English) X English (= BCI) walnut hybrids exists in the Pomology department orchards at the University of California. A smaller population of about 35 BC2 trees is also maintained there, as well as the Paradox female parent of the BCI trees and about 20 trees which are Paradox X J. hindsi. Morphological and phenological data from these trees and their seedlings is being used to estimate the heritabilities of and genotypic correlations among various vegetative traits in walnut and walnut hybrids. These traits can be used to determine which hybrids are most like English walnut. Hybrids most like English walnut have the greatest potential breeding value. Crackout data is also being used as a measure of the value of hybrids. The combined data will permit selection of seedlings for outstanding potential as a parent in the backcross breeding program.

Only hypersensitive (virus-resistant) parents can be used in the backcrossing program, so each member of the breeding pool must be tested. The BCI and most of the BC2 trees in the breeding population have been tested and the hypersensitive items identified. Testing has been done by patch-grafting infected wood into an item to be tested. If the tested item is resistant it will reject the patch-graft. If the item is not resistant the virus will infect the scion. The presence of the virus in the scion can be detected either by ELISA or by the formation of a blackline at the graft union between the tested item and its hypersensitive rootstock. The identification of molecular markers linked to GLRW hypersensitivity may lead to more rapid and accurate screening methods in the future.

A sample of DNA was taken from all the hypersensitive BCI items, as well as from their ancestors. This DNA is being evaluated for the presence of polymorphic markers which can distinguish between DNA inherited from black walnut and DNA which is from English walnut. This will allow selection of those hybrid seedlings with the greatest amount of English walnut DNA.

Controlled crosses were made to increase the size of the breeding population. Seedlings from these crosses will be evaluated using the morphological and biochemical techniques described above. Increasing the size of the backcross population increases the odds that truly exceptional backcrosses will be available.

RESULTS

The procedures for identifying DNA polymorphisms using the RAPD technique were applied to walnut for the first time in 1991. Walnut is very amenable to the procedure and about 30 useful polymorphisms are now identified. We expect that 70 or 80 polymorphisms will be sufficient for analysis of the BCI population. Selection among the BCI trees will identify BC2 families with outstanding potential. Selection can then proceed among these families using the same DNA polymorphisms. Additional polymorphisms may have to be identified for selection among elite BC2 families.

Morphological and phenological data was collected on over 400 items in 1992. Thirty-five characters were scored for each item in the BCI and BC2 populations, as well as for their parents. Similar data was taken for a
sample of Paradox, black and English walnuts and their seedlings to provide a
database for estimating trait heritabilities and genotypic correlations.
Certain characters could only be scored in mature trees, e.g., phenological
data relating to flowering. The database is now large enough to permit the
identification of combinations of vegetative characters significantly
 correlated with the percent English walnut genome present in a hybrid. By
using these combinations of variables in a multiple regression we can identify
hybrid seedlings most likely to resemble English walnut seedlings. The nuts
of the resistant BC1 trees were evaluated in the fall of 1992 and this data
was added to crackout data from the same trees taken in 1990 and 1991.

To increase the size of the breeding pool, over 1200 controlled crosses were
made in 1992. 'Chandler' is the male parent of the BC2 population, 'Sunland'
is the male parent of the BC3s. 330 BC2, 23 BC3 seeds and about 200 BC2
seedlings have been produced from these crosses. Seeds from the 1992 crosses
will be added to the breeding nursery in the spring of 1993.

Seventeen BC2 trees were identified as CLRV resistant (hypersensitive) in
1992. Most of these items have been repropagated and pruned to encourage
flower production in the coming years. The few BC2 trees that remain untested
will be patch-grafted with CLRV-infected wood in 1993 to determine if they are
hypersensitive.

CONCLUSIONS

A useful population for breeding resistance to CLRV has been established, and
evaluation has begun which will permit selection among the backcross progeny
of this population. Morphological and phenological characters useful in
selection have been identified. Biochemical analysis of the DNA of the
hybrids and their parents has shown that DNA markers can be used to identify
those hybrid seedlings which most resemble English walnut and therefore have
the best potential as parents in the breeding program. At present the best
method for eliminating backcross seedlings which are not hypersensitive to
CLRV takes at least one year from the time of grafting. The identification of
DNA markers linked to CLRV hypersensitivity could speed this process
considerably; otherwise it is unlikely that a more efficient, cost effective
method will be available in the near term. Crosses made in 1991 and 1992
are expected to produce over 200 BC2 and BC3 seedlings. Increasing the size of
the BC2 population will improve the gain from selection.