Molecular Basis of Rootstock-Scion Incompatibility in Macrophylla Decline May Reveal Useful Information For Screening Compatible Rootstock-Scion Combinations[1]

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Abstract

Several differentially expressed markers of compatibility or incompatibility were isolated and are being molecularly characterized. One marker is present in young Eureka on Macrophylla trees and on Macrophylla decline affected, Eureka on Macrophylla trees, while absent on healthy, Eureka on Macrophylla trees of the same combination. A second marker appears similar to a gene that encodes a Zn-binding homeodomain of a DNA binding protein in plant cells. This particular marker was found in the leaves of healthy trees, but absent in Macrophylla decline trees, which are known to be Zn deficient. Thirty-five markers are being characterized in all.

Introduction

Macrophylla decline (MD) and lemon sieve tube necrosis (LSTN) are decline disorders that occur in arid regions of the southwest. These disorders are major causes of substantial production loss, in western citrus. Previous research by others (Schneider, 1956; 1960; Allen, 1982) indicates that these disorders are the result of rootstock scion incompatibility. Indeed the symptoms of MD and LSTN, phloem structural abnormalities, nutrient deficiencies, reduced water conductivity, and tree decline, are similar to other incompatibilities (Allen, 1982; Taylor et al., 1995). The MD and LSTN incompatibilities are difficult to detect in that they occur late relative to other rootstock scion failures often observed prior to moving the tree from the nursery to the orchard or just as the tree becomes productive. We regard MD and LSTN as an opportunity to look for molecular markers for late onset rootstock/scion incompatibility. By establishing a set of molecular markers we will be able to produce a viable screen that may be used in future development of compatible rootstock and scion material.

We are assessing several compatible and incompatible rootstock scion pairs periodically after grafting. At these times, the graft pairs are sampled. The messenger RNA that encodes the proteins that the plant produces at the time of sampling are isolated from the plant tissues. These RNA's are employed to create a "catalogue" of the

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genes that are being expressed at that time. The genes that are expressed at that time in a given rootstock scion pair may be similar to or different from other rootstock scion pairs at the same time. We are looking for differences in gene expression that may be associated with compatibility and incompatibility. In addition we are comparing our findings from the study of the compatible and incompatible pairs with genes that are differentially expressed in healthy versus MD trees.

Materials and Methods

Field and laboratory experiments were initiated to develop material for the identification of molecular determinants of vegetative compatibility and incompatibility among rootstock-scion combinations. In addition we collected field material for similar comparisons as well as comparisons between decline and healthy trees.

Laboratory. Seeds were germinated in culture for developing the following combinations: Eureka on Cleopatra and Eureka on C-35 (incompatible combinations); Eureka on Eureka and Macrophylla on Macrophylla (autograft controls); Prior Lisbon on Macrophylla and Bessie Sweet on Macrophylla (compatible combinations); Eureka on Macrophylla (late incompatibility combination). All combinations have been approach grafted except Eureka on C-35 and Prior Lisbon on Macrophylla. The Prior Lisbon and C-35 seedlings had a great deal of contamination in culture and were started again.

Field. The following combinations have been budded and are maintained in a greenhouse in Tucson: Calamondin on Troyer, Calamondin on Sour Orange, Eureka on Cleopatra, Frost Nucellar on Sweet Orange, Eureka on C-35 (incompatible combinations); Prior Lisbon on Macrophylla and Bessie Sweet on Macrophylla (compatible combinations); Eureka on Macrophylla, Frost Nucellar on Macrophylla, and Rosenberger Lisbon on Macrophylla (late incompatibility combinations). There are also non-grafted and autografted controls, for each of the combinations above. In this study, the combinations are replicated 4 times. We will begin sampling of the bark tissues above and below the graft union from the establishment of vascular continuity. The first bark patch sample will be made 2 months post vascular continuity and at four month intervals following. We have had some difficulty establishing some combinations. Therefore not all comparisons planned for this study have been made.

Differential Display. Total RNA was isolated from most sampled combinations and their gene expression was differentially compared. RNA expression from the non-grafted controls was compared with RNA from the above many of the above field combinations. In addition, we have displayed comparisons of Macrophylla decline and healthy Rosenberger Lisbon on Macrophylla.

Results and Discussion

We have identified several potentially worthy differences in RNA expression. We have cloned thirty-five differentially expressed PCR products, and have sequenced nine. We are completing the cloning of three more and sequencing the rest. We are finding that RNA blot analysis of differential display PCR products is exceedingly difficult. We have recently worked out the complications in this process, and we just successfully completed the comparison of one set of healthy and decline comparison groups. The differential expression of a novel 400 base pair product was verified by RNA blot analysis. We continue to make such comparisons at this writing. Of the sequenced clones in hand the most interesting with regard to stress, disease and vegetative incompatibility are a 600 bp product that is expressed in the healthy but not in decline that has significant homology with the Zn-binding homeodomain of a DNA binding protein. This is of interest because we find that Zn is redistributed within the tree making it unavailable to some parts of the diseased tree. This could account for the reduced expression of a gene that encodes a Zn-binding protein. In addition, the increased expression of an NH_4^+ transporter and an oligosaccaryl transferase in an incompatible combination (Eureka on C-35) was noted in our displays, Northern analysis must be used to confirm this difference in gene expression. These comparisons have been made for leaves

and phloem tissues of mature (10 years old) trees. We anticipate that these expression differences may be manifestations of earlier changes that likely occur in these late incompatibility combinations. Therefore, we anticipate other differences in RNA expression of recently grafted late incompatibility combinations. Not only will these experiments identify potential markers of incompatibility, but they may also identify markers of Macrophylla decline of citrus.

We have identified several candidate genes that we are trying to characterize. In fact we have isolated one gene that is expressed in an incompatible pair (Eureka on Macrophylla) but is not expressed in a compatible pair (Prior on Macrophylla), and is present in MD trees but absent in healthy trees.

We know the DNA sequence of several of the genes we have isolated. This sequence gives us an idea, in some cases, as to the possible function of the genes. In other cases, the gene has never been identified before, so we do not know what it does. We will soon place several of the identified genes into citrus at excess levels in some transgenic plants and we will knock those genes out in other transgenic plants. Then we will determine what changes occur in plant growth, development, or physiology. These experiments should give us clues as to which genes we want to use to our advantage, and which ones we may want to suppress.

Literature Cited

Allen, R.M., E.L. Nigh, D.R. Rodney, and H. Schneider. 1982. Macrophylla rootstock necrosis affects more trees in Arizona. Calif. Citrograph 67: 133-134.

Schneider, H. 1960. Bud-Union trouble in Macrophylla Rootstocks of Frost Nucellar Eurekas. Calif. Citrograph 46: 2, 12.

Schneider, H. 1956. A new disorder of Eureka lemon trees. Calif. Citrograph 41: 387.

Taylor, K.C., D. R. Ellis, J.A. Gallegos, H.L. Geitzenauer. 1995. Elucidation of the physiological effects of Macrophylla decline. In: Glenn Wright ed. 1995 Citrus Research Report. Pp. 29-39.