Predicting Graft Incompatibility in Woody Plants

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We have developed a theory to explain and predict graft compatibility and incompatibility in woody angiosperms. This theory is based on the similarity of major peroxidase enzymes in the cambial tissue of stock and scion. Peroxidases mediate the production of lignins and adjacent stock and scion cells must produce similar lignins, and have identical peroxidase enzyme patterns, to ensure the development of a functional vascular system across the graft union. In some species, all, or most, individual plants produce identical peroxidase patterns, and nurserymen seldom encounter problems of graft incompatibility. In species which have traditionally been problem grafters, we have generally found variability among individuals in peroxidase enzymes. Although detailed analyses of peroxidases in such species would be the ideal method for predicting graft incompatibility, there are some steps that the practical nurseryman can take to increase grafting success.

INTRODUCTION

The grafting of fruit trees began in Biblical times and is still an accepted method of propagation of selected cultivars. Numerous scientific papers dealing with graft compatibility and incompatibility have been published and, while we still have not established biochemical or physiological criteria to predict the outcome of grafting procedures, the "trial and error" research of the past has, at least, established guidelines and expectations that fruit growers have learned to accept. Grafting research with landscape trees has been negligible, and nurserymen are still producing potentially graft incompatible combinations even when the stock and scion belong to the same species.

The following discussion is based on a series of papers that began with an exposition of the bases of the theory that peroxidase enzymes in cambial tissue were important in determining graft incompatibility (Santamour, 1988a). This was followed by detailed analyses of intraspecific grafting problems in Chinese chestnut (*Castanea mollissima* Bl.), red oak (*Quercus rubra* L.), and red maple (*Acer rubrum* L.): Santamour (1988b, 1988c, 1989). These papers were preceded by extensive generic surveys of the peroxidase enzyme patterns in 64 taxa of *Acer* (Santamour, 1982), 90 taxa of *Quercus* (Santamour, 1983), and 10 taxa of *Castanea* (Santamour et al., 1986).

GRAFT INCOMPATIBILITY

Barbara Mosse (1962) has written that "the only certain criterion of incompatibility is the characteristic interruption in cambial and vascular continuity which leads to the spectacular smooth breaks at the point of union. At the point of union no normal vascular tissue develops. The gap thus formed is filled in by proliferating ray tissue which does not lignify normally." Thus, the inability of a stock and scion to unite initially may not necessarily be a manifestation of graft incompatibility. Likewise, even if a grafted plant leaves a nursery with stock and scion "stuck together" it may still represent an incompatible combination. It may be impossible to come up with a definition of graft incompatibility that would satisfy all situations or all people. Therefore, in conversation or published papers it is important to explain the speaker's or writer's concept of graft incompatibility. In this paper, I consider long-term graft incompatibility to apply to situations where there is an initial "take" between stock and scion, but where poor growth of the scion or actual breakage at the graft union may occur after some years in the field.

ENZYMES AND LIGNIFICATION

All living plant tissues contain enzymes, special proteins that are essential catalysts for the chemical reactions leading to growth and development. Enzymes are the primary products of genes, and their analysis provides valuable data on the genetic distinctiveness of individual plants. Different tissues may contain different enzymes, and the same tissue may contain different enzymes at different stages of development. Peroxidase enzymes appear to have many diverse functions and they may be represented in any plant tissue by a number of different forms called isozymes. One exclusive function of the peroxidase isozymes is the formation of lignin(s). Lignin is the essential stabilizing component of the cell walls of woody plants. Although we often speak of "lignin" as if it were a single compound, the fact is that there are many lignins and they vary in chemical content between genera, species, and even between different tissues in an individual plant. Thus, no one can write a complete chemical formula for lignin. While it is true that the bulk of the lignin in plant cells is deposited in secondary cell walls to "strengthen" the cells, other parts of the cell, notably the middle lamella, contain appreciable quantities of lignin. Our theory of graft incompatibility (Santamour, 1988a) is based primarily on the inability of two adjacent cells with different peroxidase isoenzyme constitution to produce identical lignins in the middle lamella, thus leading to a disruption in normal cell-to-cell sap flow that will result in a non-functional vascular system.

INTRASPECIFIC GRAFTING

In developing our theory we have concentrated on certain species in which grafts between different plants of the same species could prove to be incompatible. Those species were, as noted above, Chinese chestnut, red oak, and red maple. Cambial tissue of individuals of these species varied in the production of two or three major peroxidase isozyme bands. These bands were designated, depending on their movement on the electrophoresis gels, as A, B, and C. In the most variable species we studied (red oak), all possible enzyme combinations were found among 463 different trees (A, B, C, AB, AC, or ABC), with the majority being A, B, or AB types. Only those stock and scion combinations with identical enzyme patterns were compatible (e.g. A on A, AB on AB) whereas non-identical combinations (e.g. A on AB, B on AB, AB on B) were incompatible. Similar results were obtained in intraspecific grafts of Chinese chestnut and red maple.

In some other species, notably Norway maple (*Acer platanoides* L.), sugar maple (*A. saccharum* Marsh.) and honeylocust (*Gleditsia triacanthos* L.), nurserymen and growers had never reported any great problems of graft incompatibility—and we did not find any major variations in isoperoxidase banding patterns. Thus, there appears to be a strong correlation between peroxidase variability and potential graft incompatibility. Limited work on enzyme variation in European beech (*Fagus sylvatica* L.), Goldenrain tree (*Koelreuteria paniculata* Laxm.), sycamore maple (*A. pseudoplatanus* L.), and some ashes (*Fraxinus* spp.), indicated that intraspecific grafting could lead to graft incompatibility problems.

INTERSPECIFIC AND INTERGENERIC GRAFTING

Many fruit trees, and other trees, are commonly propagated an rootstocks of different species or even different genera, and we purposely avoided the investigation of such graft combinations so that we were faced with a minimum of genetic variability in the development of our hypotheses. Among the three major genera studied (*Acer*, *Castanea*, *Quercus*), the species of *Acer* and *Quercus* exhibited considerable variability in peroxidase isozyme patterns that could be somewhat related to subgeneric or sectional botanical classification. In *Castanea*, however, individuals of all 10 species contained only the same major peroxidase isozyme bands (A,B,C), alone or in combination. In this genus, therefore, we found that graft incompatibilities between species as well as interspecific hybrids followed the same "rules" as intraspecific grafts in Chinese chestnut. Anagnostakis (1991) showed that the genes responsible for the different peroxidase bands in several chestnut species were allelic and inherited codominantly.

VIRUS-INDUCED GRAFT FAILURES

In recent years, it has been found that certain cases of "delayed graft incompatibility" do not truly represent incompatible stock-scion combinations, but are caused by viruses that pass through the stock or scion and kill the cells of one of the grafting partners at the graft union. Such situations in apple, prune, oak, and walnut are more fully discussed, and references given, in Santamour (1988a, 1988c).

HINTS FOR PROPAGATORS

What can the practical propagator do with this theory? Some suggestions are contained in papers by the author (Santamour, 1988b, 1988c, 1989) for certain species. Ideally, all stock and scion plants should be enzyme-typed before grafting, but it is unlikely that any nurserymen, and few scientists, have the equipment and time for such an undertaking. One recommendation would be to collect seed for rootstocks of a particular cultivar from that cultivar. This procedure will greatly increase the chances that a high percentage of the seedlings will have peroxidase enzyme patterns identical to that of the cultivar, but because of the unpredictability of the male parents of the seedlings, there is no guarantee of higher levels of graft compatibility. A second possibility would be to develop seed orchards composed only of enzyme-typed clones or seedlings which, with normal cross-pollination, would produce seedlings with known enzyme constitution. Such procedures may be time-consuming, and microprogagation techniques would eliminate any potential grafting problems. Still, when rootstocks with desirable characteristics such as resistance to wilt diseases or nematodes are available, grafting and the solution of incompatibility problems may be the best method of developing superior trees.

DISCUSSION

Although we believe that our new theory of graft incompatibility represents an important step in the understanding of the grafting process, it does not explain all cases of graft incompatibility.

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