Cambial Isoperoxidase Patterns in *Castanea*[1]

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Abstract

Only 3 major and variable anodal isoperoxidase bands were found in the cambial zone of 10 *Castanea* species. Even though the numbers of plants tested in several taxa were low, it appeared that certain banding patterns could be characteristic for some species. Thus, *C. dentata* and other American species (*C. alnifolia, C. ashei, C. ozarkensis, C. pumila*) exhibited only band A. The Japanese chestnut (*C. crenata*) and *C. seguinii* had only band B. The isoenzyme phenotypes A, B, and AB were found in the European chestnut (*C. sativa*) while our single tree of *C. henryii* had both A and B bands. Chinese chestnut (*C. mollissima*) contained trees with 4 isoenzyme phenotypes (A, AB, B, BC). The distribution of isoperoxidase banding patterns, coupled with widespread sexual compatibility, indicated that the categorization of chestnut species into different taxonomic sections may have little importance from a genetic basis. The possible influence of enzyme phenotype on graft compatibility is discussed.

Index words: Chestnut, enzymes, graft compatibility

Introduction

We have been studying the cambial peroxidase isoenzymes in a wide range of landscape tree genera in order to test certain of our hypotheses regarding graft incompatibility (6), mainly that plants having the same cambial peroxidase isoenzymes would be more likely to be compatible than those differing in enzyme pattern. One of the results of this research has been the often significant relationship between isoperoxidase banding patterns and various taxonomic classification schemes. Thus, in *Acer*, we were able to obtain very good correlations of isoenzyme profiles with botanical sections (7). In *Quercus*, there were significant banding pattern variations among subgenera (8).

Our present investigations in *Castanea* were undertaken because of the many reported difficulties of grafting propagation in this genus, not only at the interspecific or hybrid level, but also in intraspecific grafts within Chinese chestnut (*C. mollissima* Blume). There is a notable lack of literature on long-term grafting experiments in *Castanea*, but the situation may be best summarized by the statement of Weber and MacDaniels (10) that "Incompatibility is commonly encountered when combining stock and scion of different species of chestnut, and incompatibility may even be encountered when grafting Chinese clones on Chinese stock." In a 1-year study of budding 5 Chinese clones on seedling progeny of those clones, only 91 of 500 buds developed shoots (4). No further reports on this experiment were made, but it is likely that a further observation by Weber and MacDaniels (10), that "Grafts may make vigorous growth for several years and then die," was operable. In the United States, Chinese chestnut is the most widely planted of those foreign species resistant to chestnut blight, and many superior cultivars have been selected. In addition, because of the many interspecific hybrids that have been developed in an attempt to combat this disease, we decided to include as wide a range of material as possible in our studies.

In Camus' monograph (1), she divided the genus into 3 sections with the "chestnuts" (*C. sativa* Mill., *C. dentata* Borkh., *C. crenata* Sieb. & Zucc., *C. mollissima, C. seguinii* Dode, and *C. davidii* Dode) classified in the Section Eucastanon. Jaynes (3) later used the more modern taxonomic concepts in designating this as Section Castanea. Camus placed the "chinkapins" in 2 sections with *C. henryi* (Skan) Rehd. & Wils. in Section Hypocastanon and the remaining species (*C. pumila* Mill., *C. ashei* Sudw., *C. floridana* Ashe, *C. ozarkensis* Ashe, *C. alnifolia* Nutt., and *C. paucispina* Ashe) in the Section Balanocastanon. In addition, she noted 12 interspecific natural or artificial hybrids, many between species belonging to different sections, and erected hybrid epithets for them. Rehder (5) did not recognize any sectional classification in the genus, perhaps because of the numerous interspecific hybrids. Certainly, the work of tree geneticists (3) has shown that there are few, if any, sexual incompatibilities within the genus.

Materials and Methods

Most of the trees used in this study were growing in the test plots of the Connecticut Agricultural Experiment Station, and only the exceptions will be noted. Among native American species, we examined 70 plants of *C. dentata* grown from 2 different seed sources, grafted plants of 2 selections, and stump sprouts of 6 blighted trees at the Blandy Experimental Farm in Boyce, Virginia. Other American species were represented by far fewer trees: *C. alnifolia* (2 trees), *C. ashei* (1), *C. ozarkensis* (2), and *C. pumila* (2). Two hundred seedlings of Chinese chestnut (*C. mollissima*), 100 each from 2 different nurseries, were sampled, along with 5 trees and 6 cultivars from the Connecticut collection and 7 trees growing at the National Arboretum or in Glenn Dale, Maryland. We tested 5 trees of *C. crenata* (Japanese chestnut) and a single tree each of the Asiatic species *C. henryi* and *C. seguinii*. The European chestnut (*C. sativa*) was represented by 15 plants, 10 of which were seedlings from the cultivar 'Colossal'. A number of interspecific hybrids were also included in our analyses.

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Extracts of dormant or active cambium were prepared from 1- or 2-year-old branches by scraping with a razor blade to remove all tissues external to the xylem. Both dormant and active stem cambium, as well as root cambium, gave similar results. Because critical quantitative comparisons were judged unnecessary, roughly equal amounts of cambial tissue were placed in 0.1 ml extraction buffer in wells (5 mm × 5 mm) drilled in a solid nylon block. The extraction buffer was 1 mM tris-maleic buffer, pH 7.0, containing 5 mM potassium metabisulphite, 10 mM cysteine, and 1% (v/v) Tween 80. The samples were frozen overnight at −8° C and thawed and homogenized in the wells with an electric engraving tool. Filter paper wicks were loaded directly from the wells.

Twelve percent starch gel was prepared according to the method of Conkle (2), except that 0.14 M sucrose was added. Gels were run at 4° C using a 2.0 M lithium-borate running buffer, pH 8.0, at a constant voltage of 100 V until the bromphenol blue marker migrated 7 cm. Gels were sliced and stained for peroxidase with both 3-amino-9-ethylcarbozole (9) and 0.6 ml guaiacol and 0.5 ml 5% hydrogen peroxide in 100 ml 0.2 M acetate buffer, pH 4.0.

Results and Discussion

Only 3 major and variable anodal peroxidase bands (A, B, C) were found in the plants studied (Fig. 1). Because of their stability at various seasons of cambial collection and storage conditions, these enzymes were considered as being the most likely involved in lignin formation and lignin-carbohydrate bonding and, therefore, in grafting success. There was also some variation in cathodal bands, but Wolter and Gordon (11) have shown that cathodal peroxidases promoted growth rather than lignification.

Every individual (78 plants) of *C. dentata* had only band A (Fig. 1-E) as did all other plants of the American species *C. alnifolia* (Fig. 1-F), *C. ashei*, *C. ozarkensis*, and *C. pumila* (Fig. 1-G). All 5 trees of *C. crenata* (Fig. 1-H) and

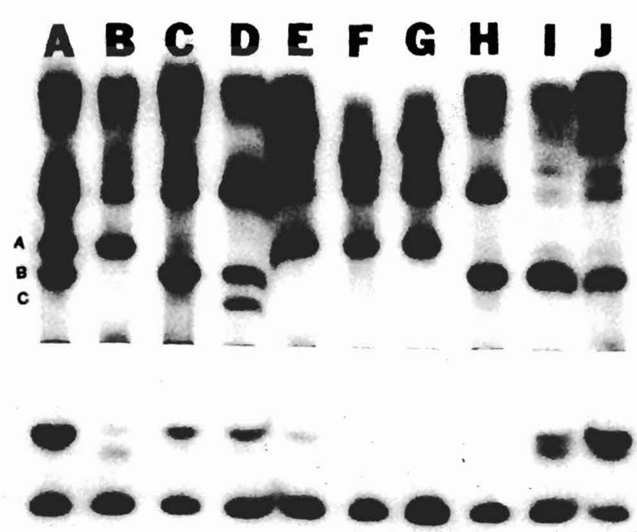

Fig. 1. **Cambial isoperoxidase banding patterns of: (A–D) *Castanea mollissima*, (E) *C. dentata*, (F) *C. alnifolia*, (G) *C. pumila*, (H) *C. crenata*, (I) *C. henryi*, (J) *C. sativa*. Major bands A, B, C, noted on left.**

the single plant of *C. seguinii* had only band B. Our only plant of *C. henryi* had both A and B bands (Fig. 1-I), with band A being much weaker than band B. In *C. sativa*, trees with both bands, singly and in combination, had phenotypes A, B, or AB, although sometimes band A was weak (Fig. 1-J).

Castanea mollissima (Fig. 1-A, B, C, D) showed the highest degree of enzyme variability of all species. Among the 200 seedlings, the following phenotypes were observed; A (5 plants), AB (98), B (89), and BC (8). Indications are that this variability was not entirely a function of the numbers of plants analyzed since we found all 4 phenotypes among the 11 trees from the Connecticut plantings and also among the 7 trees in the Washington, D.C. area.

Hybrids between species having only band A: *C. dentata* X *C. ashei* and *C. ozarkensis*; *C. alnifolia* X *C. ashei*; showed only band A. Hybrids between *C. ozarkensis* (band A) and species having AB or B bands produced both bands. Of 2 hybrids between *C. ozarkensis* (band A) and *C. mollissima*, one was A and one AB. In view of the variability of banding patterns in Chinese chestnut, such a result is entirely within reason. However, we cannot base any conclusions about inheritance on such a small sample, especially where the enzyme patterns of the individual parent trees has not been established. Such a study should be conducted in *C. mollissima*, since that species will most likely assume paramount importance among the chestnuts propagated and cultivated in the United States.

The significance of all North American species having only band A is not entirely clear. More extensive sampling might show the presence of other major enzyme bands, but the possibility of a common ancestry for all species is suggested. The lack of any really significant isoenzyme deviations from the pattern demonstrated throughout the genus indicates, as does hybridization data, that division of *Castanea* into various botanical sections is not warranted.

Much of the "literature" dealing with graft compatibility is buried in the "discussion" following presentations made at various nut-growers associations. It has been widely recognized that grafting of *C. dentata* on *C. mollissima* rootstocks has been largely unsuccessful, but that other American species graft well on *C. dentata* stocks. We were able to examine 5 young, grafted plants of a *C. dentata* selection from "Scientist's Cliffs," Maryland on *C. mollissima* rootstocks. This scion selection, like all other *C. dentata* examined, had only isoenzyme band A. Although none of the grafts were particularly vigorous, 3 were obviously near death, and these all had only band B in the root cambium. The other 2 grafts had AB rootstocks, with band A being common to both stock and scion. A 23-year-old graft of the same combination (A on AB) was still surviving in the Connecticut planting but the plant had extremely low vigor.

The Japanese report few difficulties in grafting the various cultivars of *C. crenata*, and this would seem reasonable if, as we have found, band B predominates in this species and both scion and stock have only this band. The problems in grafting *C. mollissima* cultivars on rootstocks of the same species has already been mentioned, and the wide diversity of enzyme phenotypes in Chinese chestnut might help to explain this situation. More realistic appraisals of the validity of our hypotheses will depend on the long-term evaluation of the hundreds of grafts we have made in recent years.

Significance to the Nursery Industry

This paper, and those cited in *Acer* and *Quercus*, form the basis of our studies of inter- and intraspecific variation in cambial peroxidase isoenzymes and their possible relationship to graft compatibility in these 3 genera. We have established that graft incompatibility problems in the nursery industry are most prevalent within those species (e.g. *Acer rubrum* L., *Castanea mollissima* Blume, *Quercus rubra* L.) that exhibit wide intraspecific variation in cambial peroxidase isoenzyme patterns. Similar problems would be encountered when using these variable species in interspecific grafting. When there is little or no isoperoxidase variation within a species (e.g. *Acer platanoides* L., *Gleditsia triacanthos* L.), intraspecific grafts seldom exhibit incompatibilities. Now that we have some understanding of isoenzyme variation patterns we can begin to analyze the various grafts we have made between plants of similar and different isoenzyme constitution.

Following such analyses, we may be able to provide guidance to nurserymen regarding the choice of compatible rootstocks for selected cultivars in these genera.

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Temperate Zone Woody Plants for Interior Environments[1]

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Abstract

Japanese yew (*Podocarpus macrophyllus* 'Maki') and Wheeler's Dwarf pittosporum (*Pittosporum tobira* 'Wheeler's Dwarf') grown outdoors under 3 production light levels and shade-grown fatsia (*Fatsia japonica*), dwarf gardenia (*Gardenia jasminoides* 'Radicans'), variegated pittosporum (*Pittosporum tobira* 'Variegata'), leatherleaf mahonia (*Mahonia bealei*), and Chinese mahonia (*Mahonia fortunei*) adapted well to interior conditions following production. Quality of asiatic jasmine (*Trachelospermum asiaticum*), Okinawan holly (*Ilex dimorphophylla*), and dwarf Japanese euonymus (*Euonymus japonica* 'Microphylla') grown under the 3 production light levels and all species grown in full sun, except the Japanese yew and Wheeler's Dwarf pittosporum, were judged unacceptable.

Index words: acclimatization, indoor landscaping, fatsia, dwarf gardenia, pittosporum, Japanese yew, euonymus, Asiatic jasmine, Okinawan holly, leatherleaf mahonia, Chinese mahonia

Introduction

Most plants used in interior environments are tropical or semi-tropical in nature, and adapt well to conditions of relatively low light, warm temperatures, and low humidities. Proper light acclimatization during production increases quality and survival of these plants when maintained under low interior light levels (2, 3, 4, 5, 6). Many temperate zone woody plants prefer or will tolerate low light conditions in the exterior landscape and, if adaptable, could increase the selection of plant material for the interior environment. The purpose of this study was to evaluate for interior use selected woody landscape plants typically used in the exterior landscape in the temperate zone. Plants were grown under 3 production light levels to determine the effects of light acclimatization on interior performance.

Materials and Methods

In experiment 1, 30 uniform 7.5 cm (3 in) liners each of *Fatsia japonica* (fatsia), *Gardenia jasminoides* 'Radicans'

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