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Isozymes Confirm Hybrid Parentage for 'G755' Selections

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ABSTRACT

G755 rootstocks are becoming popular because of their superior root-rot resistance. The intermediate morphological characters of G755 suggest that the rootstocks may be a product of interspecific hybridization of avocado (*Persea americana* Mill.) and coyou (*P. schiedeana* Nees), but genetically-based characters must be used to test this hypothesis. We used diagnostic isozymes from five loci to distinguish between the two putative parents. In every case, the G755 genotypes had isozymes at these diagnostic loci that combined those found solely in avocado and those found solely in coyou, confirming the hybrid origin of these rootstocks. All available evidence points to the Guatemalan race of avocado as being the most likely avocado parent of these hybrid rootstocks.

INTRODUCTION

Because of their superior resistance to avocado root-rot *(Phytophthora cinnamomi),* the G755 cultivar series is rapidly becoming the most desired avocado rootstocks in California (Brokaw, 1982; Coffey, 1985; Coffey *et al,* 1986; Zentmyer *et al,* 1977). Surprisingly, these economically important plants have not yet been identified to species. When they were first collected by Schieber in the Alta Verapaz region of Guatemala, their unusual combination of fruit, leaf, and growth characteristics suggested the possibility of inter-specific hybridization between avocado *(Persea americana* Mill.) and coyou *(P. schiedeana* Nees) (Schieber and Zentmyer, 1977; Schieber *et al,* 1984). Nonetheless, plant scientists have long known that while intermediate morphology suggests hybridization, morphology in itself is very unreliable as proof of it (Adams and Turner, 1970; Gottlieb, 1972). Thus, avocado horticulturalists have been reluctant to assign G755 interspecific species status without evidence that independently confirms the possibility *(e.g.,* Brokaw, 1982). Identifying the origin of G755 is more than an academic exercise. Obviously, producing even better rootstocks in the future will depend on understanding the genetic basis of root-rot resistance in these selections. Therefore, identifying G755's relationship to potential *Persea* parents is the first step in building the "next generation" of superior avocado rootstocks.

Recently, the genetically-based biochemical markers known as isozyme have emerged as the best characters for identifying hybrids *(e.g.,* Bell and Lester, 1978; Levin, 1975; Olivieri, 1985; Wells, 1985) because they are inherited as co-dominants fashion and are generally not altered by a plant's developmental or environmental status. In avocado,

isozymes have already proven useful as markers for cultivar identification (Torres and Bergh 1980), measuring out-crossing rates (Torres and Bergh, 1978a; Vrecenar-Gadus and Ellstrand, 1985), and confirming the origins of certain cultivar (Torres and Bergh, 1978b, 1980).

In this report, we use isozymes to test and support the hypothesis that the parentage of G755 involves both *P. americana* and *P. schiedeana—that* G755 is, in fact, an interspecific hybrid.

MATERIALS AND METHODS

Leaves from several trees of *P. americana* (all 3 races) *P. schiedeana* and G755A, G755B, and G755C were obtained from University o California collections in Riverside and at the South Coast Field Station Leaf sections of about 1 square inch were ground to a fine powder under liquid nitrogen. The powder was mixed with a 0.1 M Tris-HCl pH 8.1 buffer containing 0.1 M DTT and 12% PVP-40. The thawed extract was absorbed by filter paper wicks. Starch gel electrophoresis was performed a described by Vrecenar-Gadus and Ellstrand (1984).

A preliminary survey of 12 enzyme systems demonstrated that only four had diagnostic value in separating *P. americana* and *P. schiedeana* Namely, the fast locus of phosphoglucomutase (PGM2), the intermediate and fast loci of glutamate oxaloacetate transaminase (GOT2 and GOT3) the only locus of glutamate dehydrogenase (GDH), and the only locus o aconitase IACO). The GOT and GDH isozymes were resolved in a LiOH borate buffer system (Scandalios, 1969); the PGM and AGO isozymes were resolved in tris-EDTA-borate and morpholine-citrate buffer systems respectively (Vrecenar-Gadus and Ellstrand, 1984). Standard recipes were used for staining the isozyme bands (Wendel and Stuber, 1984).

The genetic basis of many isozyme loci have already been determined for avocado (Torres *et al.* 1978; Vrecenar-Gadus and Ellstrand, 1984). Our zymograms have been interpreted in accordance with those data. Allelic products (electromorphs) have been assigned numbers corresponding to increasing relative mobilities *(i.e.,* '2' is faster than '1'). These names an only for comparison within this paper and should not be compared to date in other avocado isozyme reports.

Table 1. Isozyme Patterns of G755 and its Putative Parents

Source (sample size*)	Isozyme patterns observed in: ACO	GDH	GOT 2	GOT3	PGM
P. americana (9) (avocado)	11, 12 & 22	22 only	11, 12 & 22	11 & 12	33 only
P. schiedeana (4) (coyou)	33 only	11 only	33 only	22 only	12 & 22
G755A	13	12	13	12	23
G755B	13	12	13	12	23
G755C	13	12	23	12	23

* Number of different selections.

RESULTS AND DISCUSSION

Table 1 lists the isozyme genotypes observed in avocado *(P. americana),* coyou *(P. schiedeana),* and the G755s. All five loci show discrete differences between avocado and coyou; *(e.g.,* PG M is monomorphic in avocado for the '3' electromorph, whereas coyou is polymorphic for the '1' and '2' electromorphs [*cf.* Torres and Bergh, 1980]). All of the G755 selections combine the diagnostic electromorphs of the two species, strongly supporting the hybrid origin of these plants. Considering that we show consistent differences over a number of genes and that the plants show intermediate characters, the possibility that G755 represents "pure" avocado or "pure" coyou is highly unlikely. The fact both species are present and common at the site of collection (Schieber and Zentmyer, 1977) further strengthens the case.

We have not absolutely ruled out the possibility that G755 represents a cross between *P. americana* or *P. schiedeana* and a third *Persea* species. This possibility is unlikely because avocado and coyou are the most common *Persea* species in the home of G755, the Alta Verapaz region of Guatemala (Schieber and Zentmyer, 1977). In fact, only a few other *Persea* species are known from the region (Schieber and Zentmyer, 1978), and all of these species have morphologies and/or isozyme patterns that are radically different from G755 (Schieber and Zentmyer, 1978; Ellstrand and Lee, unpublished data). Thus, the most parsimonious explanation for the origin of G755 is interspecific hybridization between *P. americana* and *P, schiedeana.*

Granted that G755 represents an avocado-coyou hybrid, which race of avocado is the most likely parent? The question is an economically important one. The West Indian

race is highly frost-tender and ill-adapted to cool winter soils, whereas the Mexican and Guatemalan races are sufficiently cold-hardy to be desirable parents for rootstocks in a climate like California's. Isozyme data serve fairly well in discriminating among the three races (Bergh *et al,* in preparation). The isozyme profile of G755 provides enough information to rule out the Mexican race as a parent, Unfortunately, its profile does not provide enough evidence to absolutely distinguish between the West Indian and Guatemalan races. The weight of the evidence is for the Guatemalan race because (1) G755 displays certain genes that are common in Guatemalan material and rare in West Indian (Bergh *et al*., in preparation) and (2) the West Indian race of avocado does not occur at the elevation that G755 was collected (Schieber and Zentmyer, 1978).

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