AN ABSTRACT OF THE THESIS OF

Suozhan Cheng for the degree of Doctor of Philosophy in Horticulture

presented on July 15, 1992

Title: Isozyme Variation and Inheritance in Hazelnut

Abstract approved:

Shawn A. Mehlenbacher

Inheritance of eight enzyme systems, alanine aminopeptidase (AAP), aspartate aminotransferase (AAT), aconitase (ACON), glucose phosphate isomerase (GPI), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucomutase (PGM), and shikimate dehydrogenase (SDH) was studied in hazelnut (*Corylus avellana* L.) by either polyacrylamide gel electrophoresis (PAGE) or starch gel electrophoresis. Three isozymes were detected for AAP and all were polymorphic, monomeric, and controlled by three, two, and five alleles, respectively. One zone of activity was observed for AAT and it was polymorphic; segregation data indicated that the active form of the enzyme is a dimer and is controlled by a single locus with two alleles. Two isozymes were identified for ACON. Both were polymorphic, monomeric, and controlled by two loci with three alleles each. Two variable and one monomorphic regions of activity were observed for GPI; *Gpi*-2 behaved as a dimer and was controlled by a single locus

with two alleles; *Gpi*-3 was also controlled by a single locus with two alleles but was active as a monomer. Three MDH isozymes were identified and one zone was polymorphic; *Mdh*-1 was active as a dimer and was controlled by a single locus with two alleles, although a deficiency of aa phenotypes was detected. Two zones of activity were observed for 6-PGD, one of which was polymorphic, active as a dimer, and controlled by a single locus with three alleles. Two zones of activity were identified for PGM; both were polymorphic, monomeric, and controlled by two separate loci with three and four alleles, respectively. Two isozymes were observed for SDH, one of which was polymorphic, monomeric, and controlled by a single locus with four alleles. Rare alleles were detected at *Aat, Aco*-1, *Gpi*-2, *Mdh*-1, and *Sdh*-2.

Of the 17 isozyme loci studied, 13 were polymorphic. The most useful enzyme systems for hazelnut cultivar identification were AAP, PGM, ACON, and GPI. 54 of 56 cultivars studied could be distinguished by a combination of the isozymes examined. Thus, isozyme analysis is a very powerful tool for identifying hazelnut cultivars. 'Tombul' and 'Extra Ghiaghli' appear to be the same cultivar based on both isozyme analysis and morphological characteristics.

Three linkage groups were detected. 6-*Pgd*-2 was tightly linked to the leaf anthocyanin gene ($0 \pm 0.06$) in three crosses. *Gpi*-2 and *Mdh*-1 were linked with a recombination frequency of $6.86 \pm 2.55$ in two progenies. A loose linkage was found among *Aco*-1, *Pgm*-2, and *Pgm*-1.

# ISOZYME VARIATION AND INHERITANCE IN HAZELNUT

by

Suozhan Cheng

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed July 15, 1992

Commencement June 1993

APPROVED:

Associate Professor of Horticulture in charge of major

Head of Department of Horticulture

Dean of Graduate School

Date thesis is presented July 15, 1992

Typed by Suozhan Cheng

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my major professor Dr. Shawn Mehlenbacher for his guidance, encouragement, and support during my graduate studies.

My appreciation is also directed to Dr. Tony Chen, Dr. Patrick Breen, Dr. Patrick Hayes, Dr. Porter Lombard, Dr. Warren Kronstad, and Dr. James Vomocil (graduate representative) for their support and for serving on my graduate committee. Special thanks to Dr. Tony Chen for allowing me to use his lab equipment. Thanks are also extended to Dr. Thomas Adams for allowing me to use his starch gel trays and to David Smith for his assistance during my research.

I also would like to thank many individuals for their help and technical advice in new techniques. Dr. Aaron Liston gave me a lot of technical advice and allowed me to do a project using Polymerase Chain Reaction in his lab. Dr. Benhui Liu assisted me in using his computer program. Dr. Fuqiang Chen taught me the methods of identifying RFLP markers. I would also like to thank all my friends for their encouragement and help throughout my studies.

Finally, I want to thank my wife for her understanding, encouragement, and support. And thanks to my son for making our life more enjoyable.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF APPENDIX TABLES

# ISOZYME VARIATION AND INHERITANCE IN HAZELNUT

## Chapter 1

## INTRODUCTION

Hazelnuts, members of the genus *Corylus,* belong to the Birch family, Betulaceae. They are deciduous trees and shrubs distributed through temperate regions of the northern hemisphere. The number of species ranges from 7 to 20 depending upon the taxonomic authority. The 9 most widely recognized include 5 shrubby species and 4 tree species. All species produce edible nuts, which vary considerably in the quality of the kernels, the shell thickness, and the ease of extracting nuts from the husks. All *Corylus* species that have been investigated for chromosome numbers are diploid with 2n=22 (Mehlenbacher, 1991).

*Corylus avellana* is the most economically important species in the genus *Corylus*. This species is native to Europe and Asia Minor where it is a common shrub in the forest understory. All important world cultivars were selected directly from this species, which is very rich in genetic diversity. *C. avellana* exhibits tremendous variability in plant size and growth habit, nut size, nut shape, husk length, and many other morphological traits. Although the European hazelnut is native to areas with different climatic extremes, the world's major production countries are limited to only a few areas near large bodies of water. All are characterized by mild, humid winters and cool summers (Mehlenbacher, 1991).

Worldwide, there are nearly 400 distinct, named cultivars (Mehlenbacher,

1991). Some cultivars in collections are mislabelled while other cultivars are represented several times under different names because the germplasm was introduced from different places. Unfortunately, detailed descriptions are not available for most cultivars. Traditional identification is usually based on morphological traits. Isozymes may provide a reliable and simple alternative method for identifying hazelnut cultivars.

Public hazelnut breeding programs were initiated in the 1960s in Italy and Oregon and in the 1970s in France and Spain. 11 quantitative traits and 5 simply inherited traits have been identified (Thompson et al., 1992). Simply inherited traits include leaf color, style color, self-incompatibility, nondormancy, and immunity to eastern filbert blight ( Mehlenbacher and Thompson, 1988; Mehlenbacher and Thompson, 1991; Mehlenbacher et al., 1991). Only one linkage group has been found in hazelnut; Thompson (1985) showed that red pigmentation was closely linked to the S locus.

Isozyme analysis has proven to be a powerful tool for estimating genetic diversity in germplasm collections, identifying cultivars and selections, studying the inheritance of qualitative and quantitative traits, and determining linkage relationships to aid in the construction of genetic maps (Weeden, 1989). Genetic differences among cultivars may be reflected in allozyme polymorphisms, and isozyme phenotypes have been used to distinguish cultivars for many crops (Moore, 1984; Peffley et al., 1985; Weeden, 1986; Weeden and Lamb, 1985). As a consequence of the simple genetic control of isozyme variation and the lack of

environmental influence in their expression (Tanksley and Orton, 1983), these markers have also been used for inheritance and linkage studies in many crops, including field crops (Goodman et al., 1980; Griffin and Palmer, 1987), vegetables (Vallejos and Chase, 1991), and tree fruits (Parfitt and Arulsekar, 1989; Weeden and Lamb, 1987).

The objectives of this research were (a) to characterize important hazelnut cultivars and parents used extensively in the Oregon State University breeding program using isozyme polymorphism, (b) to determine the inheritance of these isozyme markers and (c) to look for possible linkages among isozyme loci and between isozyme loci and traits of economic interest.

# Chapter 2

# LITERATURE REVIEW

## The Techniques of Isozyme Analysis

The term isozyme was coined by Markert and Moller (1959) to describe different molecular forms of enzymes with the same substrate specificity. Strictly defined, isozymes are different molecular forms of an enzyme coded by more than one locus, and allozymes are the products of different alleles at the same locus (Gottlieb, 1982).

The application of electrophoresis to determine the presence of various forms of an enzyme was an important development in plant genetics. In general, electrophoresis allows the separation of different proteins extracted from plant tissues. The process is carried out by running an electric charge through a supporting medium (usually either a starch or polyacrylamide gel) onto which the protein has been placed. The proteins are allowed to migrate for a specific amount of time and then are stained with various chemicals so that the relative mobility of specific proteins can be determined. Relative mobility is a function of the size and charge of the molecule. If two proteins have different amino acid sequences, they often have different mobilities because the differences in sequence result in a change in size and/or charge of the molecule (Hedrick, 1983).

Two main types of supporting media, polyacrylamide and starch, are used for

separating isozymes. Polyacrylamide gel electrophoresis (PAGE) was introduced by Raymond and Weinstraub (1959). PAGE has very good resolving power, for the gels can be poured uniformly and provide greater flexibility in the sieving properties, stacking systems, and assay compatibility. Many polyacrylamide systems include a stacking gel in which the sample is concentrated into a very thin band before entering the resolving gel (Weeden, 1989). The disadvantages for using PAGE are that (1) acrylamide is a neurotoxin, (2) the apparatus is much more expensive than that for starch gels, (3) the gels are not easily sliced, and only one assay can be performed on a gel (Weeden, 1989).

Starch is often preferred to polyacrylamide for reasons that include simplicity of gel preparation, the non-toxic properties of the gel components, lower equipment costs, and ease of sample preparation. Perhaps the most important reason for using starch gels is that they can be sliced horizontally into a number of duplicate slabs, each of which may be assayed for a different enzyme system (Weeden, 1989).

## Advantages and Disadvantages of Isozymes

Isozymes have proven to be useful genetic markers in different research fields. They can be used in nearly every organism and many loci can be examined. Their expression is usually independent of environmental conditions or the tissues sampled and the variability detected is close to the DNA level. Isozymes are simply inherited, codominant, and do not exhibit pleiotropy or

epistasis (Hedrick, 1983; Weeden, 1989). However, isozyme markers have the following disadvantages relative to other types of molecular markers. Isozyme variation consists of only protein-coding loci. Some genetic variation may not be identifiable. Some amino acid substitutions may not result in changes in charge. Enzyme systems may reveal different degrees of genetic variability. Some variants are expressed only in certain tissues or life stages (Ayala, 1975; Hedrick, 1983; Lewontin, 1974). For example, *Acp*-1, a locus linked to the pale green lethal gene (I) in apple, is only expressed in the cotyledons (Manganaris and Alston, 1988b).

## Protein Extraction in Woody Species

Different tissues, including leaves, bark, roots, and flowers, can be used for protein extraction for isozyme studies. In most cases, leaves are used because they can be easily collected without sacrificing young seedlings. A difficulty in tree species is the presence of varying amounts of phenolic compounds in most tissues which interfere with enzyme activity. Appropriate extraction techniques generally must be developed in order to obtain satisfactory results (Torres, 1983).

The major interaction to be controlled is that between proteins and phenols, and their oxidation products (Anderson and Sowers, 1968; Dirr et al., 1972). Plant phenolics range from simple monomers to large polymers: two phenylpropanoid compounds (including hydrolyzable tannins) and the flavonoids (including condensed tannins). Tannins form strong hydrogen bonds with the peptide linkage of proteins, through the oxygen of the peptide bond. Phenols can

also oxidize to quinones. Quinones polymerize easily and form covalent bonds with the -SH and -$NH_2$ groups of proteins (Loomis and Battaile, 1966).

The free sulphydryl groups of proteins are highly reactive, and when oxidized, may form disulfide bonds and cause enzyme inactivation. Many compounds have been used to prevent protein-phenol interaction during extraction. Reducing agents, such as ascorbate, cysteine, 2-mercaptoethanol, and dithiothreitol (DTT) are included in the extraction buffers to prevent the oxidation of phenols and the accumulation of quinones (Loomis and Battaile, 1966; Tucker and Fairbrothers, 1970).

Some compounds, such as polyvinylpyrrolidone (PVP) and polyvinylpolypyrrolidone (PVPP), contain groups similar to the peptide bonds of protein and may effectively bind phenols. When PVP or PVPP is added during extraction, phenols react with the PVP and form complexes which can be removed by centrifugation (Dirr et al. 1972). Denaturing agents such as urea should not be included when extracting with PVP because they cause the PVP-phenol complex to dissociate (Anderson and Sowers, 1968).

Some authors have suggested that preparations be made as acetone powders (Sanderson, 1964) in order to remove phenols from samples. Bendall and Gregory (1963) used 80% acetone except for final drying of the precipitation with pure acetone. Though the preparation of acetone powders separates most phenols and enzyme inhibitors from the protein fraction, the addition of reducing agents is still necessary to prevent enzyme inactivation (Tucker and Fairbrothers, 1970).

Controlling the pH of extraction buffers is essential for maximum recovery of protein and enzyme activity. The optimum pH for extraction depends on the particular enzyme. The optimum pH for the activity of the enzyme, however, is not necessarily that at which the enzyme is most stable (Cooper, 1977). The pH of most buffers for extraction of proteins in fruit crops ranges from 7-8.5 (Arulsekar and Parfitt, 1986; Byrne and Littleton, 1988; Durham et al., 1987; Roose and Traugh, 1988; Weeden and Lamb, 1985).

## Cultivar Identification

Isozyme analysis has been used for germplasm diversity studies in many field crops, vegetables, and fruits (Tanksley and Orton, 1983; Weeden, 1989). Isozyme markers have been used for cultivar identification in many fruit crops (Table 2-1). Traditional cultivar identification is based in morphological characteristics, some of which vary widely with the environment. Isozyme phenotypes, however, are consistent within a genotype and most are independent of rootstock, age, and environment (Vinterhalter and James, 1983; Weeden and Lamb, 1985). This technique is comparatively economical and applicable in small laboratories as well as in large scale operations (Weeden and Lamb, 1985)

The choice of enzymes which show variation among cultivars is very important for their successful use in cultivar identification. In apple, the enzyme systems used for distinguishing cultivars are peroxidase, acid phosphatase, and esterase (Korban and Bournival, 1987; Menendez et al., 1986; Vinterhalter and

James, 1983), and 6-phosphogluconate dehydrogenase (6-PGD), aspartate aminotransferase (AAT), diaphorase, and isocitrate dehydrogenase (IDH) (Weeden and Lamb, 1985). Of these, the most useful isozymes studied were 6-PGD and AAT (Weeden and Lamb, 1985). More than 10 isozyme systems have been examined in peach. Most were monomorphic among the cultivars surveyed and only malate dehydrogenase (MDH) showed great variation (Arulsekar et al., 1986; Durham et al., 1987). Recently, Mowrey et al. (1990) found variation in two additional enzymes, IDH and shikimate dehydrogenase (SDH) in a peach cultivar collection. Byrne and Littleton (1988) examined eight enzyme systems in plum. The isozymes which showed differences among cultivars were glutamate dehydrogenase (GDH), leucine aminopeptidase (LAP), MDH, glucose phosphate isomerase (GPI), phosphoglucomutase (PGM), and peroxidase, but 6-PGD and triosephosphate isomerase (TPI) were not variable. In grape, Stavrakakis and Loukas (1983) reported that esterase, LAP, secondarily peptidase, GDH, and PGM had the greatest number of differences among cultivars. Hauagge et al. (1987b) found the most useful enzymes for almond cultivar identification to be AAT, GPI, LAP, and PGM. In addition, catalase (CAT) and acid phosphatase (ACP) were also useful (Cerezo et al., 1989). GPI and PGM showed great variation in persimmon (Sugiura et al., 1988). AAT, PGM, and GPI had the best discriminating power in kiwifruit, and all the New Zealand cultivars were uniquely identified by the simultaneous comparison of these three enzyme systems (Messina et al., 1991).

Isozyme studies have demonstrated that some species are much more genetically variable than others. Apples are highly polymorphic and most cultivars can be distinguished on the basis of 2 enzyme systems (Weeden and Lamb, 1985). The banding patterns for three enzymes revealed greater diversity in *Corylus* than in *Pyrus* (Menendez and Daley, 1986; Ahmad et al., 1987). Grapes are also highly polymorphic, as 37 cultivars could be identified using 2 enzyme systems (Stavrakakis and Loukas, 1983). Peach and walnut do not appear to be highly polymorphic (Arulsekar et al., 1986; Cheng and Yang, 1987). At best, the cultivars studied could only be classified into several groups.

Many reports have shown that isozymes are useful in determining the parentage of cultivars and the relationships among them. In almond, historical records and isozyme similarities revealed the existence of an original pool of seedlings selected before 1900, and most recent cultivars were hybrids of two important cultivars (Hauagge et al., 1987b). Evidence for the probable parentage of some pineapple cultivars was provided by isozyme banding patterns (Dewald et al., 1988). In mango, reported parentage of some cultivars was inconsistent with their isozyme banding patterns (Degani and El-Batsri, 1990). Sports of fruit cultivars are generally indistinguishable from the original cultivars, (Menendez et al., 1986; Weeden and Lamb, 1985). Isozyme analysis provides guidance to nurserymen regarding the choice of compatible rootstocks for *Castanea* cultivars (Santamour et al., 1986).

## Genetics and Linkage

Isozymes have been used extensively as genetic markers in plants (Tanksley and Orton, 1983; Weeden and Wendel, 1989), including many fruit crops (Table 2-2).

Apple has been extensively investigated (Cheng, 1982; Chevreau and Laurens, 1987; Manganaris and Alston, 1987, 1988a and b, 1992a and b; Weeden and Lamb, 1987). Manganaris and Alston (1992b) identified 8 polymorphic loci for peroxidase and found two linkage groups: *Prx*-2 and *Prx*-3, and *Prx*-4 and *Prx*-5. Weeden and Lamb (1987) identified 19 distinct isozyme loci and 3 linkage groups.

In peach, MDH is inherited in a simple Mendelian manner, but one phenotype is absent in commercial cultivars (Arulsekar et al., 1986; Durham et al., 1987; Mowrey and Werner, 1990). About 15 isozyme loci have been studied and two linkage groups identified in blueberry (Heemstra et al., 1991; Vorsa et al., 1988).

The codominant expression of isozyme loci renders them well-suited for detecting linkage with each other and with morphological traits because heterozygous and homozygous genotypes can be easily distinguished. Linkages between isozyme loci and important traits have been found in many crops (Table 2-3).

Since most fruit trees require an extended juvenile phase before fruiting, the identification of linkages between isozyme markers and genes affecting fruit quality

or other traits not expressed until the adult phase would be very useful to breeders. This would allow indirect selection for some trait at the seedling stage. In fig (*Fiscus carica* L.), a linkage relationship between an isozyme locus (*Prx*-3) and the gene for sex determination (Valizadeh, 1978) could greatly enhance the efficiency of fig breeding programs. Manganaris and Alston (1987, 1988b, and 1992b) established several linkage groups in apple. A close linkage between *Got*-1 and the incompatibility locus was revealed by the differing arrays of Got-1 phenotypes in reciprocal progenies of apple (Manganaris and Alston, 1987). Two genes, *Acp*-1 and *Enp*-1, were identified as closely linked to the pale green lethal gene (*l*) in apple in the order of *Acp*-1--*Enp*-1--*l* (Manganaris and Alston, 1988b). Manganaris and Alston (1992a) also found a close linkage between *Lap*-2 and a mildew resistance gene derived from 'White Angel'. In peach, Werner and Moxley (1991) found an association between *Mdh*-1 and plant vigor. Plant vigor was significantly less in *Mdh*1-1--*Mdh*1-1 homozygotes; homozygous *Mdh*1-2--*Mdh*1-2 individuals showed the greatest vigor; and heterozygotes were intermediate in vigor.

Linkages between isozymes and morphological traits have been reported in several other species. Isozymes linked with self-incompatibility have been detected in *Nicotiana alata* (Labroche et al., 1983), *Plantago lanceolata* (Van Dijk, 1985), rye (Wricke and Wehling, 1985; Gert and Wricke, 1989), and tomato (Tanksley and Loaiza-Figueroa, 1985). In garden pea, resistance to Fusarium wilt (race 1) is linked to an esterase locus (Hunt and Barnes, 1982). In bean, resistance to bean

yellow mosaic virus is closely linked to *Pgm-p* (Weeden et al., 1984), and resistance to pea enation mosaic is linked to *Adh*-1 (Weeden and Provvidenti, 1987). In rice, a shikimate dehydrogenase locus (*Sdh*1-2) is associated with seed protein content. The mean protein content per seed was highest for *Sdh*1-2 homozygotes, intermediate for *Sdh*1-1--*Sdh*1-2 heterozygotes, and the lowest for *Sdh*1-1 homozygotes (Shenoy et al., 1990).

Table 2-1. Cultivar identification by isozyme analysis in fruit crops.

| Crop | Reference |
|---|---|
| Almond | Cerezo et al. 1989 |
| | Hauagge et al. 1987 |
| Apple | Cheng and Jia 1981 |
| | Chyi and Weeden 1984 |
| | Menendez et al. 1986 |
| | Vinterhalter and James 1983 |
| | Weeden and Lamb 1985 |
| Apricot | Byrne and Littleton 1989 |
| Avocado | Goldring et al. 1985 |
| Banana | Jarret and Litz 1986 |
| Chestnut | Anagnostakis 1991 |
| | Santamour et al. 1986 |
| Citrus | Anderson et al. 1991 |
| | Roose and Traugh 1988 |
| | Soost et al. 1980 |
| | Torres 1982 |
| Fig | Valizaedeh 1977 |
| Grape | Chaparro et al. 1989 |

Table 2-1. Cultivar identification by isozyme analysis in fruit crops (Contd.).

| Crop | Reference |
|---|---|
| | Parfitt and Arulsekar 1989 |
| | Schwennesen et al. 1982 |
| | Stavrakakis and Loukas 1983 |
| | Subden et al. 1987 |
| Hazelnut | Ahmad et al. 1987 |
| Kiwifruit | Li et al. 1986 |
| | Messina et al. 1991 |
| | Zhu and Lawes 1987 |
| Mango | Degani and El-Batsri 1989 |
| Olive | Pontikis et al. 1980 |
| Papaya | Moore and Litz 1984 |
| Peach | Arulsekar et al. 1986 |
| | Durham et al. 1987 |
| | Messeguer et al. 1987 |
| | Mowrey et al. 1990 |
| Pear | Lin and Shen 1983 |
| | Menendez and Daley 1986 |
| | Santamour and Demuth 1980 |

Table 2-1. Cultivar identification by isozyme analysis in fruit crops (Contd.).

| Crop | Reference |
|---|---|
| Pecan | Marquard 1987 |
| | Mielke and Wolfe 1982 |
| Persimmon | Sugiura et al. 1988 |
| | Tao and Sugiura 1987 |
| Pineapple | Dewald et al. 1988 |
| Plum | Byrne and Littleton 1988 |
| Strawberry | Arulsekar and Bringhurst 1983 |
| Walnut | Arulsekar et al. 1985 |
| | Cheng and Yang 1987 |

Table 2-2. Number of isozyme loci and linkage groups in fruit crops.

| Crop | Enzyme studied (number of loci) | Linkage groups | Reference |
|---|---|---|---|
| Apple | PRX (4) | | Cheng et al. 1984 |
| | LAP (4) | | Manganaris and Alston 1992a |
| | PRX (8) | *Prx*-2---*Prx*-3, *Prx*-4--*Prx*-5 | Manganaris and Alston 1992b |
| | ACP(1), ADH (2), ENP (1), EST (1) | | |
| | GOT (2), GPI (1), IDH (1), and PGM (1) | | Chevreau and Laurens 1987 |
| | *Got*-2 and *Got*-4 | *Got*-2--*Lap*-2 | Manganaris and Alston 1988a |
| | *Acp*-1 and *Enp*-1 | *Acp*-1--*Enp*-1 | Manganaris and Alston 1988b |
| | AAT (2), DIA (5), GPI (3), IDH (2) | *Aat-c*--*Idh*-1, *Gpi*-c2--*Aat*-p, | |
| | MDH (5), ME (1), PGM (5), and TPI (4) | (*Dia*-5, *Pgm*-p1)--(*Mdh*-2, *Tpi*-c2) | Weeden and Lamb 1987 |
| Almond | AAT (2), ACP (2), GAP (2), GPI (2), | | |

Table 2-2. Number of isozyme loci and linkage groups in fruit crops (Contd.).

| Crop | Enzyme studied (number of loci) | Linkage groups | Reference |
|---|---|---|---|
| | LAP (2), PGM (2), and 6-PGD (2) | | Hauagge et al. 1987 |
| Avocado | LAP (2), MDH (1), PGM (2), and PRX (3) | | Torres et al. 1980 |
| Blueberry | IDH (1), GPI (2), MDH (2), and 6-PGD (2) | | Vorsa et al. 1988 |
| | ACO (2), ADH (2), ALD (1), G-3-PDH (2), | | |
| | IDH (1), LAP (1), MDH (3), 6-PGD (1), | *Pgi*-2--*Lap*-1, *Pgm*-2--6-*Pgd*-2 | |
| | PGI (2), and PGM (2) | | Heemstra et al. 1991 |
| Grape | ALD (1), ICD (1), FUM (1), G-6-PD (1), GPD (1), | | |
| | PEP (2), PGI (1), PGM (1), and TO (1) | | Loukas et al. 1983 |
| | AAT (4), EST (5), GPI (2), LAP (1), | *Acp*-1--*Pgm*-c, *Acp*-2--*Aat*-c, | |
| | MDH (4), PRX (2), and PGM (2) | *Gpi*-c--*Lap*-1 | Weeden et al. 1988 |

Table 2-2. Number of isozyme loci and linkage groups in fruit crops (Contd.).

| Crop | Enzyme studied (number of loci) | Linkage groups | Reference |
|---|---|---|---|
| | GPI (2) and PGM (2) | | Parfitt and Arulsekar1989 |
| Citrus | GOT (2), HK (1), IDH (1), LAP (2), | *Got*-1--*Mdh*-1, *Mdh*-2--*Me*-2 | |
| | MDH (2), ME (2), PGI (1), PGM (1), SOD (1) | | Torres et al. 1985 |
| Peach | MDH (1) | | Arulsekar et al. 1986 |
| | MDH(1) and PRX (1) | | Durham et al. 1987 |
| | IDH (1), MDH (2), and SDH (1) | | Mowrey and Werner 1990 |
| | CAT (1) | | Werner 1992 |
| Pecan | MDH (1), PGI (2), and PGM (1) | | Marquard 1987 and 1991 |
| Walnut | AAT (2) and GPI (2), | | Arulsekar et al. 1985 |
| | EST (3) and PGM (3) | | Arulsekar et al. 1986 |

Table 2-2. Number of isozyme loci and linkage groups in fruit crops (Contd.).

| Crop | Enzyme studied (number of loci) | Linkage groups | Reference |
|---|---|---|---|

Enzyme abbreviations: ACP, acid phosphatase; ACO, aconitase; ADH, alcohol dehydrogenase; ALD, aldolase; AAT, aspartate aminotransferase; DIA, diaphorase; ENP, endopeptidase; EST, esterase; FUM, fumarase; G-6-PD, glucose-6-phosphate dehydrogenase; G-3-PDH, glyceraldehyde-3-phosphate dehydrogenase; GPI, glucosephosphate isomerase; HK, hexokinase; IDH, isocitrate dehydrogenase; LAP, leucine aminopeptidase; MDH, malate dehydrogenase; ME, malic enzyme; 6-PGD, 6-phosphogluconate dehydrogenase; PGM, phosphoglucomutase; PRX, peroxidase; SDH, shikimate dehydrogenase; SOD, superoxide dismutase; TO, tetrazolium oxidase.

Table 2-3 Isozyme loci linked or associated with morphological traits in fruit and other economically important crops.

| Crop | Isozyme locus | Linked trait | Reference |
|---|---|---|---|
| Apple | *Lap*-2 | Mildew resistance | Manganaris and Alston 1992a |
| | *Acp*-1 and *Enp*-1 | Pale green lethal gene *I* | Manganaris and Alston 1988b |
| | *Got*-1 | Self incompatibility | Manganaris and Alston 1987 |
| Peach | *Mdh*-1 | Plant vigor | Werner and Mowley 1991 |
| Fig | *Prx*-3 | Sex determination | Valizadeh 1978 |
| Bean | *Adh*-1 and *Got*-2 | Affecting seed size | Vallejos and Chase 1991 |
| Celery | *Mdh*-2 | Annual habit (*Hb*) | Quiros et al. 1987 |
| Lentil | *Aat-p* | Green epicotyl (*Gs*) | Muehlbauer et al. 1989 |
| | *Pgm-p* | Cotyledon color (*Yc*) | Muehlbauer et al. 1989 |
| Lettuce | *Est*-8 | Downy mildew resistance (*Dm*-1) | Landry et al. 1987 |

Table 2-3. Isozyme loci linked or associated with traits in fruit and other economically important crops (Contd.).

| Crop | Isozyme locus | Linked trait | Reference |
|---|---|---|---|
| Nicotiana | *Prx*-1 | Self-incompatibility | Labroche et al. 1983. |
| Pea | *Pgm-p* | Bean yellow mosaic virus resistance (*Mo*) | Weeden et al. 1984 |
| | *Adh*-1 | Pea enation mosaic resistance (*En*) | Weeden and Provvidenti 1987 |
| | *Est* | Fusarium (race 1) resistance (*Fw*) | Hunt and Barnes 1982 |
| Rice | *Sdh*-1 | High seed protein content | Shenoy et al. 1990 |
| Rye | *Prx*-7 | Self incompatibility | Wricke and Wehling 1985 |
| Soybean | *Idh*-1 | Affecting stem development (*F*) | Hedges et al. 1990 |
| | *Idh*-1 | Regulating nodulation (*Rj*1) | Hedges et al. 1990 |
| | *Aco*-2 | *Rj*2 | Devine et al. 1991 |
| | *Idh*-2 | High stearic acid level (*Fas*) | Rennie and Tanner 1989 |

Table 2-3. Isozyme loci linked or associated with traits in fruit and other economically important crops (Contd.).

| Crop | Isozyme locus | Linked trait | Reference |
|---|---|---|---|
| | *Idh*-2 | Low linoleic acid level (*Fan*) | Rennie and Tanner 1989 |
| Squash | *Ald*-2 | Watermelon mosaic virus-2 resistance | Weeden et al. 1984 |
| Sugarbeet | *Idh*-2 | Red hypocotyl color (*R*) | Smed et al., 1989 |
| Tomato | *Acp*-1 | Nematode resistance (mi) | Rick and Fobes 1974 |
| | *Idh*-1 | Self-incompatibility | Tanksley and Loaiza-Figueroa 1985 |
| | *Prx*-1 | Self-incompatibility | Tanksley and Loaiza-Figueroa 1985 |
| | *Prx*-2 | Male sterility (Ms) | Tanksley et al. 1984 |
| | *Pgi*-1 | Cold tolerance | Vallejos and Tanksley 1983 |

Chapter 3

# INHERITANCE AND LINKAGE OF ASPARTATE AMINOTRANSFERASE, MALATE DEHYDROGENASE AND GLUCOSE PHOSPHATE ISOMERASE IN HAZELNUT

## Abstract

Inheritance of aspartate aminotransferase (AAT), malate dehydrogenase (MDH), and glucose phosphate isomerase (GPI) was studied in hazelnut (*Corylus avellana* L.) by either polyacrylamide or starch gel electrophoresis. One zone of activity was observed for AAT and it was polymorphic. Segregation ratios indicated that the active form of the enzyme is a dimer, with two alleles present at a single locus. Three zones of activity were observed for MDH and GPI and polymorphisms were detected in one and two zones, respectively. Segregation ratios indicated that *MDH*-1 is dimeric and is controlled by a single locus with two alleles, although a deficiency of aa genotypes was detected. *GPI*-2 behaved as a dimeric enzyme with two alleles . *GPI*-3 was also controlled by a single locus with two alleles but was active as a monomer. Rare alleles were detected at *Aat*, *Mdh*-1, and *Gpi*-2 among the cultivars studied. Cosegregation in two progenies indicated a tight linkage between *Mdh*-1 and *Gpi*-2.

## Introduction

Hazelnut (*Corylus avellana* L.) is one of the world's major nut crops, ranking second to almond in world production. The crop is grown in the areas near large bodies of water, where all are characterized by mild, humid winters and cool summers (Mehlenbacher, 1991).

Little information is available on inheritance of traits in hazelnut. Only five simply inherited traits have been identified, including eastern filbert blight immunity, nondormancy, incompatibility, red leaf color, and yellow leaf color in 'Barcelona' seedlings (Thompson et al., 1992). Isozymes are useful genetic markers because of their lack of physiological effects and their codominant expression. Isozymes have been used as genetic markers in a wide variety of investigations, including screening of germplasm for new sources of variation, identification of cultivars and hybrids, genetic analysis, marking genes controlling commercially important traits, studying evolution, and analyzing somaclonal variation (Weeden, 1989). The objectives of this research were to determine the inheritance of three enzyme systems, AAT, MDH and GPI, and to determine linkage of isozyme loci.

## Materials and Methods

Young seedlings for this study were grown in a greenhouse or at the Smith Horticulture Farm, and were derived from controlled crosses made in 1988, 1989, and 1990 at the farm, as part of the Oregon State University (OSU) hazelnut breeding program. Young leaves were collected from actively growing shoots at the greenhouse and the farm and carried to the lab for immediate use. Leaf tissue (0.05 g) from each seedling was diced and ground with 1 ml of cold extraction buffer in a chilled mortar. The extraction buffer was tris-citrate (pH 8.0), containing 0.1% 2-mercaptoethanol and 3% soluble polyvinlpyrolidone (PVP-40). The macerated sample was transferred to a microcentrifuge tube, which was kept on ice, and centrifuged at 10,000 rpm. The supernatant was used immediately for electrophoresis.

Both polyacrylamide gel electrophoresis (PAGE) and starch gel electrophoresis were used. Starch gels were made as described by Arulsekar and Parfitt (1986) and Cardy et al. (1980). Polyacrylamide gels were made as described by Cheng and Yang (1987). Electrophoresis was carried out at a constant 200v for PAGE (tris-HCl, pH 8.8), and at a constant 100v for both tris-citrate histidine (pH 5.7), and citrate histidine starch gels (pH 5.7). PAGE gels were stained for AAT; starch gels at pH 7.0 snd 5.7 were stained for GPI and MDH, respectively. The staining procedures were modified from Arulsekar and Parfitt (1986) and Cardy et al. (1980).

In systems with multiple loci, the locus with the greatest anodal migration was designated as 1; slower migrating loci were assigned progressively higher numbers. Alleles at each polymorphic locus were similarly designated alphabetically. Goodness-of-fit tests were performed using the computer program G-Mendel (Liu and Knapp, 1990). Expected ratios were 1:1 for progenies exhibiting two phenotypes and 1:2:1 for those exhibiting three phenotypes. Linkage analyses were performed using the same program (user specified model).

## Results and Discussion

*Aspartate aminotransferase* (AAT) AAT exhibited one cathodic zone of activity (Fig. 3-1 and 3-2). Segregating progenies exhibited either two or three phenotypes. Of the 15 segregating progenies investigated, 14 exhibited two phenotypes in a ratio of 1:1. The other segregating progeny exhibited three phenotypes in the ratio 1:2:1. In progenies exhibiting two phenotypes, the single-banded phenotype was designated bb and the triple-banded phenotype was designated ab. Progenies exhibiting three phenotypes were two single-banded (aa and bb) and one triple-banded (ab). When two bb parents were crossed, no segregation was observed in the progeny. The segregation patterns indicated that *Aat* is a dimeric enzyme coded by two alleles. G statistics for goodness-of-fit were nonsignificant (Table 3-1). AAT has been found to be dimeric in other species (Arulsekar et al, 1985; Hauagge et al, 1987).

The a allele was found to be rare in the OSU collection. In a survey of 78 cultivars and selections, no aa homozygotes and only 12 heterozygotes were observed (Chapter 5). In this study, aa homozygotes were obtained in the expected proportion in a cross between two heterozygotes.

*Malate dehydrogenase* (MDH) MDH exhibited three zones of activity (Fig. 3-1 and 3-3). *Mdh*-1 was polymorphic but *Mdh*-2 and *Mdh*-3 were monomorphic. Segregation ratios indicated that *Mdh*-1 is a dimeric enzyme coded by two alleles. When a single-banded (bb) parent was crossed with a triple-banded parent (ab), the

seedlings showed the parental phenotypes in a 1:1 ratio. G-statistics were nonsignificant for 15 of the 17 progenies (Table 3-2). When two triple-banded parents were crossed, three phenotypes, two single-banded (aa and bb) and one triple-banded (ab) phenotypes were observed in the seedlings. The expected ratio was 1aa:2ab:1bb. Both progenies exhibited a deficiency of aa phenotypes (Table 3-2). When two bb parents were crossed, no segregation was observed in the progeny. MDH has been found to be dimeric in other plant species (Mowrey and Werner, 1990; Vorsa et al., 1988; Weeden and Lamb, 1987).

In a survey of 78 cultivars and selections, no aa homozygotes and only 16 heterozygotes were observed (Chapter 5). Homozygotes of the aa type were only identified in the progenies of crosses between triple-banded phenotypes (ab). Similar results were observed for MDH in peach (Arulsekar et al, 1986; Durham et al., 1987; Mowrey et al., 1990). In peach, *Mdh*-1-1 homozygotes were shown to be less vigorous than heterozygotes and *Mdh*-1-2 homozygotes (Werner and Mowrey, 1991). A relationship between MDH and plant vigor was not observed in two hazelnut progenies in which trunk caliper and tree heights were measured ('Negret' x 'Tonda di Giffoni' and '255-3' x 'Tonda di Giffoni'). One possible explanation is that the a allele may be tightly linked to a lethal or sublethal gene and thus eliminated through selection, either by nature or by breeders. Secondly, 'Tonda di Giffoni', one of the parents in the two crosses, was found to be partially male-sterile because of a reciprocal translocation (Salesses and Bonnet, 1988), which may cause segregation distortion.

*Glucose phosphate isomerase* (GPI) GPI exhibited three zones of activity (Fig. 3-1). *Gpi*-1 was often diffuse and was not analyzed genetically. *Gpi*-2 had single- (aa or bb) and triple-banded phenotypes (ab) in the progenies. Segregation data indicated that *Gpi*-2 was a single locus governing a dimeric enzyme coded by two alleles. In a survey of 78 cultivars and selections, only one aa phenotype (GH 184-19) was found for *Gpi*-2 and the heterozygotes (ab) (3) were much less frequent that bb homozygous phenotypes (74) (Chapter 5). In the 'Tonda di Giffoni' (ab) x 'Segorbe' (ab) cross, aa homozygotes were obtained in the expected proportion, but the sample size was small. However, in bb x ab crosses, an apparent deficiency of ab phenotypes was observed in five of six progenies (Table 3-3). *Gpi*-2 may be linked to an undesirable character and the a allele in both heterozygous and homozygous forms eliminated through selection.

For *Gpi*-3, in aa x ab crosses, the pooled G estimate was significant ($p=0.04$) and aa phenotypes were deficient. In ab x ab and ab x bb crosses, the pooled goodness-of-fit estimates were not significant. In bb x bb cross, no segregation was observed in the progeny (Table 3-3). *Gpi*-3 was a monomer coded by two alleles. An interlocus designated *Gpi*-x was observed between locus *Gpi*-2 and *Gpi*-3 (Fig. 3-1). In 'Tonda di Giffoni' x 'Segorbe', 'Brixnut' x 'Segorbe', '243-2' x 'Tonda di Giffoni', 'Tonda di Giffoni' x 'GH 184-19', and 'Tombul' x 'Tonda di Giffoni' crosses, cosegregation of *Gpi*-x and *Gpi*-3 was observed. The results were not scored because of overlapping between *Gpi*-x and *Gpi*-3. Presence of *Gpi*-x was always associated with presence of the a allele at *Gpi*-2.

*Linkage Studies* Linkage for *Gpi*-2--*Mdh*-1 was detected in 'Negret' x 'Tonda di Giffoni' and '255-3' x 'Tonda di Giffoni', and the recombination frequency from the pooled data was 6.86±2.55 (Table 3-4). *Gpi*-2 and *Mdh*-1 might be linked to the same undesirable trait because these two loci were closely linked.

In our studies, we found that some alleles, such as the a alleles at *Aat*, *Mdh*-1, and *Gpi*-2, were rare and heterozygous phenotypes (ab) were much less frequent than homozygous phenotypes (bb) among the cultivars studied. Homozygous phenotypes of the aa type were only identified in the progenies of crosses between triple-banded phenotypes (ab).

Table 3-1. Single locus segregation and goodness-of-fit for *Aat*.

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 255-3 x Tonda di Giffoni | ab x ab | 24aa:47ab:29bb | 1:2:1 | 0.86 | 0.66 |
| Tonda di Giffoni x 55-77 | ab x bb | 13ab:10bb | 1:1 | 0.40 | 0.53 |
| 17-75 x Redleaf #3 | ab x bb | 52ab:54bb | 1:1 | 0.15 | 0.70 |
| Tonda di Giffoni x GH 184-19 | ab x bb | 11ab:9bb | 1:1 | 0.20 | 0.75 |
| Pendula x Pellicle Rouge | ab x bb | 8ab:7bb | 1:1 | 0.07 | 0.80 |
| Tonda di Giffoni x 243-2 | ab x bb | 13ab:10bb | 1:1 | 0.22 | 0.64 |
| Waterloo x 226-118 | ab x bb | 26ab:22bb | 1:1 | 0.33 | 0.57 |
| Tombul Ghiaghli x Hall's Giant | ab x bb | 17ab:19bb | 1:1 | 0.11 | 0.74 |
| Casina x Redleaf #3 | ab x bb | 21ab:23bb | 1:1 | 0.08 | 0.78 |
| Willamette x Tonda di Giffoni | bb x ab | 11ab:12bb | 1:1 | 0.04 | 0.84 |

Table 3-1. Single locus segregation and goodness-of-fit for *Aat* (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Ribet x Tombul Ghiaghli | bb x ab | 14ab:16bb | 1:1 | 0.12 | 0.73 |
| Negret x Tonda di Giffoni | bb x ab | 37ab:59bb | 1:1 | 5.09 | 0.02* |
| 296-82 x VR 8-32 | bb x ab | 10ab:10bb | 1:1 | 0 | 1.00 |
| Tombul x Tonda di Giffoni | bb x ab | 12ab:10bb | 1:1 | 0.17 | 0.68 |
| G pooled | | 245ab:261bb | 1:1 | 0.51 | 0.48 |
| G heterogeneity | | | | 6.47 | 0.89 |
| 43-91 x Imperiale de Trebizonde | bb x bb | 48bb | --- | --- | --- |
| Sant Jaume x 55-77 | bb x bb | 20bb | --- | --- | --- |

Table 3-2. Single locus segregation and goodness-of-fit for *Mdh*-1.

| Cross | Parental phenotype | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tonda di Giffoni x 55-77 | ab x bb | 10ab:13bb | 1:1 | 0.40 | 0.53 |
| Negret x 225-77 | ab x bb | 20ab:18bb | 1:1 | 0.40 | 0.53 |
| Tonda di Giffoni x GH 184-19 | ab x bb | 10ab:7bb | 1:1 | 0.53 | 0.47 |
| Tonda di Giffoni x Segorbe | ab x bb | 11ab:10bb | 1:1 | 0.05 | 0.83 |
| Waterloo x 226-118 | ab x bb | 23ab:25bb | 1:1 | 0.08 | 0.77 |
| Pellicle Rouge x Pendula | ab x bb | 9ab:6bb | 1:1 | 0.60 | 0.44 |
| Casina x Redleaf #3 | ab x bb | 24ab:20bb | 1:1 | 0.36 | 0.55 |
| Romai x 54-39 | ab x bb | 13ab:8bb | 1:1 | 1.19 | 0.27 |
| Tonda Romana x 23-17 | ab x bb | 15ab:11bb | 1:1 | 0.62 | 0.43 |
| Tonda Romana x 23-24 | ab x bb | 11ab:18bb | 1:1 | 1.71 | 0.19 |

Table 3-2. Single locus segregation and goodness-of-fit for *Mdh*-1 (Contd.).

| Cross | Parental phenotype | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tonda Romana x TGDL[z] | ab x bb | 18ab:7bb | 1:1 | 5.00 | 0.03* |
| 162-71 x Hend 5-5 | bb x ab | 14ab:6bb | 1:1 | 3.29 | 0.07 |
| 39-44 x Imperiale de Trebizonde | bb x ab | 15ab:9bb | 1:1 | 1.50 | 0.22 |
| 43-91 x Imperiale de Trebizonde | bb x ab | 16ab:32bb | 1:1 | 5.30 | 0.02* |
| 243-2 x Tonda di Giffoni | bb x ab | 7ab:9bb | 1:1 | 0.25 | 0.62 |
| Tombul x Tonda di Giffoni | bb x ab | 14ab:9bb | 1:1 | 1.09 | 0.30 |
| Fusco Rubra x Romisondo G-1 | bb x ab | 10ab:14bb | 1:1 | 0.67 | 0.40 |
| G pooled | | 240ab:222bb | 1:1 | 0.70 | 0.40 |
| G heterogeneity | | | | 22.34 | 0.13 |

Table 3-2. Single locus segregation and goodness-of-fit for *Mdh*-1 (Contd.).

| Cross | Parental phenotype | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 17-75 x Redleaf #3 | bb x bb | 106bb | --- | --- | --- |
| Sant Jaume x 55-77 | bb x bb | 20bb | --- | --- | --- |
| Negret x Tonda di Giffoni | ab x ab | 17aa:44ab:35bb | 1:2:1 | 7.62 | 0.02* |
| 255-3 x Tonda di Giffoni | ab x ab | 18aa:52ab:30bb | 1:2:1 | 3.28 | 0.20 |
| G pooled | | 35aa:96ab:65bb | 1:2:1 | 9.21 | 0.01** |
| G heterogeneity | | | | 1.69 | 0.43 |

[z]Tonda Gentile delle Langhe

Table 3-3. Single locus segregation and goodness-of-fit for *Gpi*-2 and *Gpi*-3.

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| | *Gpi* - 2 | | | | |
| Fusco Rubra x Segorbe | bb x ab | 9ab:14bb | 1:1 | 1.08 | 0.30 |
| Negret x Tonda di Giffoni | bb x ab | 45ab:51bb | 1:1 | 0.38 | 0.54 |
| 255-3 x Tonda di Giffoni | bb x ab | 56ab:44bb | 1:1 | 1.44 | 0.23 |
| Brixnut x Segorbe | bb x ab | 2ab:9bb | 1:1 | 4.80 | 0.03* |
| 243-2 x Tonda di Giffoni | bb x ab | 7ab:11bb | 1:1 | 0.89 | 0.34 |
| Tombul x Tonda di Giffoni | bb x ab | 6ab:17bb | 1:1 | 5.48 | 0.02* |
| G pooled | | 125ab:146bb | 1:1 | 1.63 | 0.20 |
| G heterogeneity | | | | 12.44 | 0.03* |

Table 3-3. Single locus segregation and goodness-of-fit for *Gpi*-2 and *Gpi*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tonda di Giffoni x GH 184-19 | ab x aa | 7aa:10ab | 1:1 | 0.53 | 0.47 |
| Tonda di Giffoni x Segorbe | ab x ab | 5aa:11ab:5b | 1:2:1 | 0.04 | 0.98 |
| 39-44 x Imperiale de Trebizonde | bb x bb | 24bb | --- | --- | --- |
| Fusco Rubra x 55-129 | bb x bb | 23bb | --- | --- | --- |
| | *GPI* - 3 | | | | |
| Negret x Tonda di Giffoni | aa x ab | 38aa:58ab | 1:1 | 4.20 | 0.04* |
| 255-3 x Tonda di Giffoni | aa x ab | 42aa:58ab | 1:1 | 2.56 | 0.11 |
| Sant Jaume x 55-77 | aa x ab | 13aa:13ab | 1:1 | 0 | 1 |

Table 3-3. Single locus segregation and goodness-of-fit for *Gpi*-2 and *Gpi*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 243-2 x Tonda di Giffoni | aa x ab | 7aa:11ab | 1:1 | 0.89 | 0.34 |
| Romai x 54-39 | aa x ab | 10aa:11ab | 1:1 | 0.05 | 0.83 |
| Willamette x 23-17 | aa x ab | 18aa:15ab | 1:1 | 0.27 | 0.60 |
| TGDL[z] x Mortarella | aa x ab | 10aa:13ab | 1:1 | 0.39 | 0.53 |
| 30-13 x Rode Zeller | ab x aa | 11aa:12ab | 1:1 | 0.04 | 0.84 |
| Pellicle Rouge x Pendula | ab x aa | 8aa:7ab | 1:1 | 0.07 | 0.80 |
| Tombul Ghiaghli x Hall's Giant | ab x aa | 13aa:10ab | 1:1 | 0.39 | 0.53 |
| Casina x Fusco Rubra | ab x aa | 11aa:11ab | 1:1 | 0 | 1 |
| 23-17 x TGDL | ab x aa | 31aa:39ab | 1:1 | 0.92 | 0.34 |

Table 3-3. Single locus segregation and goodness-of-fit for *Gpi*-2 and *Gpi*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G pooled | | 212aa:258ab | 1:1 | 4.52 | 0.04* |
| G heterogeneity | | | | 5.26 | 0.92 |
| | | | | | |
| 39-44 x Imperiale de Trebizonde | ab x bb | 12ab:12bb | 1:1 | 0 | 1 |
| Casina x USOR 14-82 | ab x bb | 8ab:13bb | 1:1 | 1.19 | 0.28 |
| Casina x USOR 24-82 | ab x bb | 11ab:11bb | 1:1 | 0 | 1 |
| Imperiale de Trebizonde x 43-91 | ab x bb | 27ab:21bb | 1:1 | 0.75 | 0.39 |
| USOR 24-82 x Purple Aveline | bb x ab | 16ab:17bb | 1:1 | 0.03 | 0.86 |
| G pooled | | 74ab:74bb | 1:1 | 0 | 1 |
| G heterogeneity | | | | 1.97 | 0.74 |

Table 3-3. Single locus segregation and goodness-of-fit for *Gpi*-2 and *Gpi*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Casina x Redleaf #3 | ab x ab | 7aa:24ab:13bb | 1:2:1 | 2.00 | 0.33 |
| Tombul x Tonda di Giffoni | ab x ab | 5aa:13ab:5bb | 1:2:1 | 0.40 | 0.52 |
| Contorta x Redleaf #3 | ab x ab | 2aa:6ab:2bb | 1:2:1 | 0.40 | 0.52 |
| G pooled | | 14aa:43ab:20bb | 1:1:1 | 2.10 | 0.35 |
| G heterogeneity | | | | 0.70 | 0.96 |
| Willamette x self | aa x aa | 23aa | --- | --- | --- |

[z]Tonda Gentile delle Langhe

Table 3-4. Linkage and recombination frequency for *Mdh*-1 and *Gpi*-2 loci.

| Locus pair | Cross | Number of progeny | G | P | Recombination value ± SE |
|---|---|---|---|---|---|
| *Mdh*-1/*Gpi*-2 | Negret x Tonda di Giffoni | 96 | 44.35 | .00 | 11.31±4.59 |
| | 255-3 x Tonda di Giffoni | 100 | 56.24 | .00 | 2.08±2.03 |
| | Pooled | 196 | 97.64 | .00 | 6.86±2.55 |

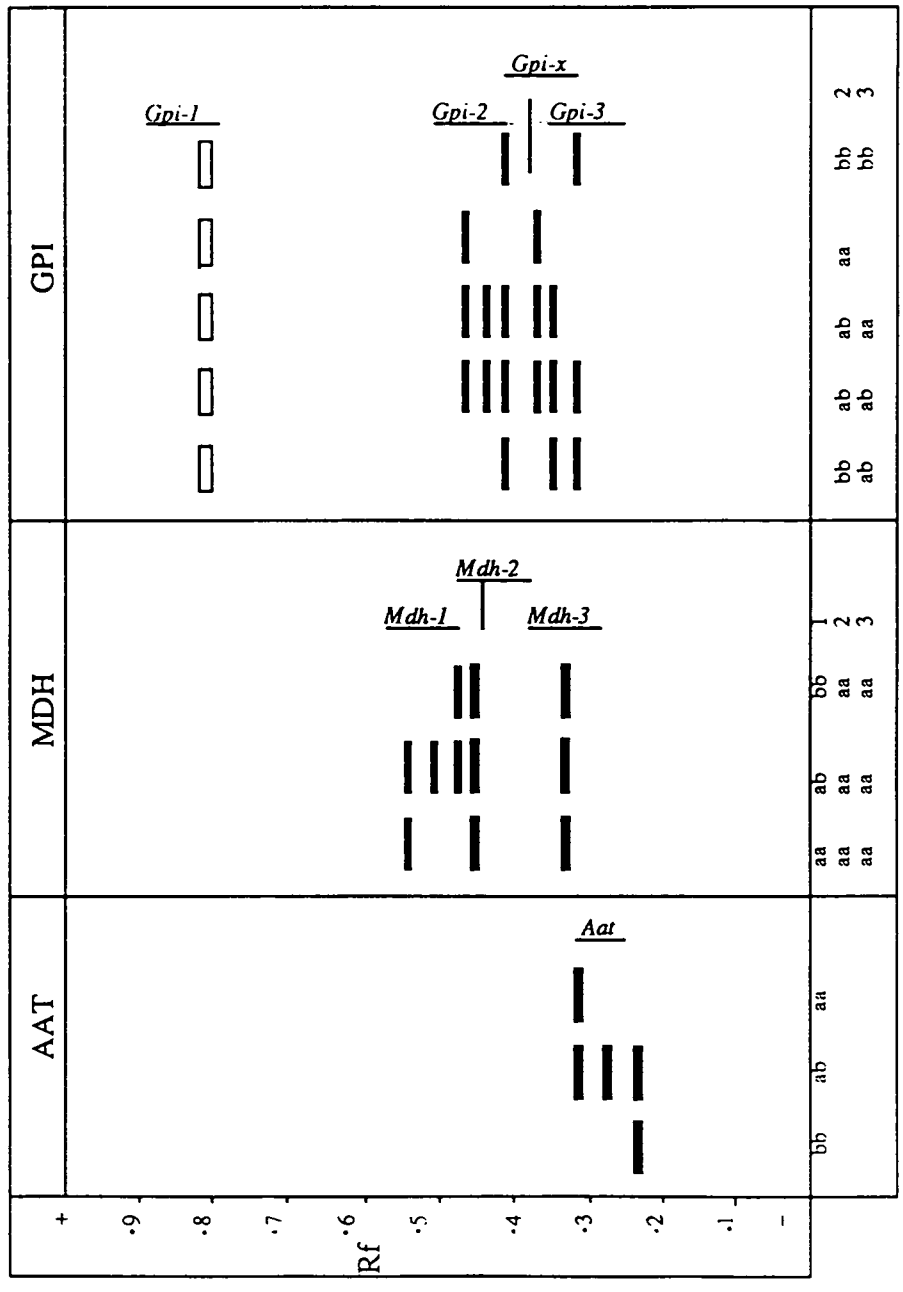


Fig. 3-1. Schematic presentation of allelic variation of AAT, MDH, and GPI in hazelnut.

Fig. 3-2. AAT phenotypic variation in the progeny from '255-3' x 'Tonda di Giffoni'.

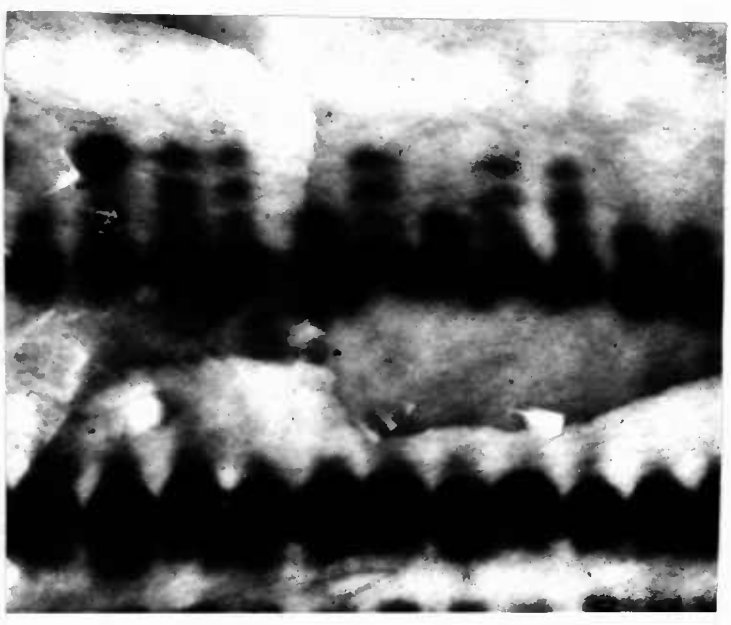

Fig 3-3. MDH phenotypic variation in the progeny from 'Negret' x 'Tonda di Giffoni'.

## Literature Cited

Arulsekar, S., D. E. Parfitt, and G. H. McGranahan. 1985. Isozyme gene markers in *Juglans* species. Inheritance of GPI and AAT in *J. regia* and *J. hindsii*. J. Hered. 76:103-106.

Arulsekar, S. and D. E. Parfitt. 1986. Isozyme analysis procedures for stone fruit, almond, grape, walnut, pistachio, and fig. HortScience 21:928-933.

Arulsekar, S., D. E. Parfitt, W. Beres, and P. E. Hansche. 1986. Genetics of malate dehydrogenase isozymes in the peach. J. Hered. 77:49-51.

Cardy, B. J., C. W. Stuber, and M. M. Goodman. 1980. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Inst. Stat. Mim. Ser. 1317, North Carolina State Univ., Raleigh.

Cheng, S. Z. and W. H. Yang. 1987. Taxonomic studies of ten species of genus *Juglans* based on isozymic zymograms. Acta Hort. 14:90-96.

Durham, R. E., G. A. Moore, and W. B. Sherman. 1987. Isozyme banding patterns and their usefulness as genetic markers in peach. J. Amer. Soc. Hort. Sci. 112:1013-1018.

Hauagge, R., D. E. Kester, and R. A. Asay. 1987. Isozyme variation among California almond cultivars: 1. Inheritance. J. Amer. Soc. Hort. Sci. 112:689-698.

Liu, B. H. and S. J. Knapp. 1990. G-MENDEL: a program for Mendelian

segregation and linkage analysis of individual or multiple progeny populations using log-likelihood ratios. J. Hered. 81:407.

Mehlenbacher, S. A. 1991. Hazelnuts, p. 789-836. In: J. N. Moore and J. R. Ballington (eds.), Genetic resources of temperature fruit and nut crops. Acta Hort. 290.

Mowrey, B. D., D. J. Werner, and D. H. Byrne. 1990. Inheritance of isocitrate dehydrogenase, malate dehydrogenase, and shikimate dehydrogenase in peach and peach x almond hybrids. J. Amer. Soc. Hort. Sci. 115:312-319.

Salesses, G. and A. Bonnet. 1988. Cytogenetic study of hybrids between hazelnut variaties (*Corylus avellana*) carrying a translocation in heterozygous state. (in French). Cytologia 53:407-413

Thompson, M. M., H. B. Lagerstedt, and S. A. Mehlenbacher. 1992. Hazelnuts. In: J. Janick and J. N. Moore (eds.), Advances in Fruit Breeding, Vol. 2. (in press).

Vorsa, N., P. S. Manos, and van Heemstra, M. I. 1988. Isozyme variation and inheritance in blueberry. Genome 30:776-781.

Weeden, N. F. and R. C. Lamb. 1987. Genetics and linkage analysis of 19 isozyme loci in apple. J. Amer. Soc. Hort. Sci. 112:865-872.

Weeden, N. F. 1989. Applications of isozymes in plant breeding. Plant Breeding Reviews 6:11-39.

Werner D. J. and D. F. Moxley. 1991. Relationship between malate

dehydrogenase isozyme genotype and plant vigor in peach. J. Amer. Soc. Hort. Sci. 116:327-329.

Chapter 4

# INHERITANCE OF ACONITASE, ALANINE AMINOPEPTIDASE, PHOSPHOGLUCOMUTASE, SHIKIMATE DEHYDROGENASE, AND 6-PHOSPHOGLUCONATE DEHYDROGENASE IN HAZELNUT

## Abstract

Inheritance of aconitase (ACON), alanine aminopeptidase (AAP), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), and shikimate dehydrogenase (SDH) in hazelnut (*Corylus avellana* L.) was studied by either polyacrylamide or starch gel electrophoresis. Two zones of activity were observed for 6-PGD, one of which was polymorphic, active as a dimer, and controlled by a single locus with three alleles. All other enzymes were active as monomers. Two variable regions of activity were identified for ACON, with three alleles present at each locus. Three isozymes were detected for AAP and all were polymorphic and controlled by three, two, and five alleles, respectively. Two zones of activity were observed for PGM, both were polymorphic and coded by loci with 3 and 4 alleles, respectively. Two isozymes were identified for SDH, one of which was polymorphic, with four alleles present at a single locus. Analysis of cosegregation indicated a tight linkage between 6*Pgd*-2 and the leaf anthocyanin gene in three crosses and a loose linkage among *Aco*-1,*Pgm*-2, and *Pgm*-1.

## Introduction

Hazelnut (*Corylus avellana* L.) is an important crop in Oregon, where 98% of the U. S. crop is produced. Five simply inherited traits have been identified, including eastern filbert blight immunity, nondormancy, incompatibility, red leaf color, and yellow leaf color in seedlings of 'Barcelona'. Heritability estimates have been calculated for 11 quantitative traits, including big bud mite susceptibility, date of budbreak, husk length, kernel volume, percent kernel, kernel shrinkage, kernel fiber, number of nuts/cluster, nut weight, shell thickness, and pellicle removal (Thompson et al., 1992). Only one linkage group, that of red leaf color and the S-locus, has been identified in hazelnut (Thompson, 1985).

Isozymes have been used as genetic markers in some fruit and nut tree crops, including apple (Chevreau et al., 1985; Chevreau and Laurens, 1987; Manganaris and Alston, 1988a and b; Weeden and Lamb, 1987), almond (Hauagge et al., 1987), grape (Loukas et al., 1983; Weeden et al., 1988; Parfitt and Arulsekar, 1989), peach (Arulsekar et al., 1986; Durham et al., 1987; Mowrey et al., 1990), and blueberry (Vorsa et al., 1988; van Heemstra et al., 1991). Linkage between isozyme loci and self-incompatibility genes has been found in tomato (Tanksley and Loaiza-Figueroa, 1985), rye (Wricke and Wehling, 1985), and apple (Manganaris and Alston, 1987). The objectives of this study were to examine the genetic basis of five enzyme systems in hazelnut and look for possible linkages among isozyme loci and between isozyme loci and morphological traits.

## Materials and Methods

The young seedlings for this study were grown in the greenhouse and at the OSU Smith Farm, and were derived from controlled crosses made in 1988, 1989 and 1990. Three crosses segregating for red and green leaves were used to investigate linkage of isozymes and leaf color. Young leaves were collected and carried to the lab for immediate use. Leaf tissue (0.05 g) from each seedling was diced and ground with 1 ml of cold extraction buffer in a chilled mortar. The extraction buffer was tris-citrate (pH 8.0), containing 0.1% 2-mercaptoethanol and 3% soluble polyvinlpyrolidone (PVP-40). The macerated sample was transferred to a microcentrifuge tube, which was kept on ice, and centrifuged at 10,000 rpm. The supernatant was used immediately for electrophoresis.

Both polyacrylamide gel electrophoresis (PAGE) and starch gel electrophoresis were used. Starch gels (11%) were made as described by Arulsekar and Parfitt (1986) and Cardy et al. (1980); starch was from Connaught Labs, Ontario, Canada. PAGE gels were made as described by Cheng and Yang (1987). Electrophoresis was carried out at a constant 200v for PAGE (tris-HCl, pH8.8), and at a constant 100v for both tris-citrate histidine (pH 7.0), and citrate histidine starch gels (pH5.7). PAGE gels were stained for AAP and SDH; starch gels at pH 7.0 and 5.7 were stained for ACON and PGM, and 6-PGD, respectively. The staining procedures were modified from Arulsekar and Parfitt (1986) and Cardy et al. (1980).

For enzymes with multiple loci, the locus with the greatest anodal migration was designated as 1; slower migrating loci were assigned progressively higher numbers. Alleles at each polymorphic loci were similarly designated alphabetically. When no activity was observed, the allele was designated null. Statistical analyses were performed using the computer program G-MENDEL (Liu and Knapp, 1990). Expected ratios were 1:1 for progenies exhibiting two phenotypes, 1:2:1 for those exhibiting three phenotypes, and 1:1:1:1 for those exhibiting four phenotypes. Linkage analyses were performed using the same program (user specified model).

## Results

Good results were obtained with all five enzyme systems. Of the 10 loci observed, 9 loci were polymorphic and studied genetically.

*Aconitase* (ACON) Two zones of activity, designated ACON-1 and ACON-2, were detected for ACON. Both zones were polymorphic. For ACON-1, when two-banded parents were crossed with single-banded parents, the parental phenotypes were recovered in the progeny in equal proportions (Table 4-1). Three and four classes of progenies were obtained from bc x bc and bc x ac crosses, respectively. In the bc x cc, ac x cc and bc x bc crosses, the G-statistics for the pooled ratio were not significant. In the ac x bc and bb x bc crosses, the G-statistics were not significant for pooled goodness-of-fit, but were significant for heterogeneity. One bb x bb cross was examined and no segregation was observed in the progeny. ACON-1 was monomeric and under the control of a single locus (*Aco*-1) with three alleles (Fig. 4-1, Table 4-1). In a survey of 78 cultivars and selections, no aa homozygotes and only three heterozygotes (ac), one cultivar ('Tonda Gentile delle Langhe') and two selections ('55-77' and '55-129'), were observed (Chapter 5). 'Tonda Gentile delle Langhe' is one of parents of the two selections. In the bc x ac crosses, an apparent deficiency of a_ phenotypes (heterozygotes with a allele) was observed in one of three progenies.

For ACON-2, the b allele was present in all parents and seedlings. Goodness-of-fit to expections was obtained in all segregating progenies. In bb x bb cross, no

segregation was observed in the progeny. ACON-2 was monomeric and under the control of a single locus (*Aco*-2) with three alleles (Fig. 4-1 and Table 4-1). In the 78 cultivars and selections investigated, only 'Tonda Romana' had the a allele. In ab x bb crosses, however, the expected ratio was obtained in all progenies. ACON has been found to be monomeric in other plant species (Doong et al., 1987; Hyun et al., 1987; Heemstra et al., 1991).

*Alanine aminopeptidase* (AAP) Three zones of activity, designated AAP-1, AAP-2, and AAP-3, were observed. For AAP-1, segregation ratios were in agreement with the interpretation of a monomeric enzyme designated *Aap*-1 with three alleles (Fig. 4-1 and Table 4-2). For AAP-2, the banding patterns for this zone were either one-banded or two-banded in the progeny, and showed monomeric structure. The G-statistics for pooled goodness-of-fit and heterogeneity for AAP-2 were not significant. In the one bb x bb cross examined, no segregation was observed in the progeny. The locus was designated as *Aap*-2 and two alleles were observed (Fig. 4-1 and Table 4-2). For AAP-3, in cd x bd, cd x cd, bd x bd, bc x cd, dd x bd and ac x cd crosses, the G-statistics for pooled data and heterogeneity were not significant. In ac x dd crosses, a highly significant deficiency of ad genotypes was detected (P=0.003), but heterogeneity was not significant. In '43-91' (bc) x 'Imperiale de Trebizonde' (bd), highly significant deviation from the expected ratio of 1:1:1:1 was found. In the one dd x dd cross examined, no segregation was observed in the progeny. Segregation data showed that this zone is monomeric (Fig. 4-1 and Table 4-2). AAP-3 was designated as

*Aap-3* with five alleles. Four alleles were observed in this study. AAP was found to be a monomer in *Cryptomeria japonica* (Tsumura et al., 1989).

*Phosphoglucomutase* (PGM) Two PGM zones of activity were observed. For PGM-1, homozygotes and heterozygotes showed single- and double-banded phenotypes, respectively. In aa x ab, ab x ab, and ab x bb crosses, the G-statistics for pooled data and heterogeneity were not significant. In aa x nn, bb x nn, aa x bb, and bb x bb crosses, no segregation was observed in the progenies. PGM-1 was a monomer and designated *Pgm*-1 with three alleles: a, b, and n (Fig. 4-1 and Table 4-3). For PGM-2, in ac x cc and aa x ab crosses, G-statistics for pooled data and heterogeneity were not significant. In aa x ac crosses, a highly significant deficiency of aa phenotypes was observed (P=0.008), but heterogeneity for PGM-2 were not significant. In aa x nn, cc x aa, and nn x nn crosses, no segregation was detected in the progenies. PGM-2 was a monomer and designated *Pgm*-2 with four alleles (Fig. 4-1 and Table 4-3). PGM has been found to be monomeric in other plant species (Hauagge et al., 1987; Hyun et al., 1987; Weeden and Lamb, 1987).

*Shikimate dehydrogenase* (SDH) Two isozymes were observed for SDH. One band between alleles a and b was found in all cultivars, selections, and seedlings and was designated *Sdh*-1. For SDH-2, all crosses except 'USOR 24-82' x 'Purple Aveline' showed good fit to expectation. SDH-2 was controlled by a single locus designated *Sdh*-2 with four alleles (Fig. 4-1 and Table 4-4). Other

plant species have been shown to possess a monomeric structure for SDH (Pitel et al., 1987; Tsumura et al., 1989; Mowrey et al., 1990).

*6-Phosphogluconate dehydrogenase* (6-PGD) Two isozyme zones of activity were observed for 6-PGD. 6-PGD-1 was monomorphic. For 6-PGD-2, in the 'Ribet' x 'Segorbe' cross, three phenotypes, two single-banded and one triple-banded, were observed. In single-banded phenotypes, the band stained intensively. The three bands were lightly stained in triple-banded phenotypes. G statistics for goodness-of-fit to 1:1 and 1:2:1 ratios were nonsignificant (Fig. 4-1 and Table 4-5). The results indicated that triple-banded individuals were heterozygous and single-banded individuals were homozygous. 6-PGD-2 was dimeric and was controlled by a single locus (*6-Pgd-2)* with three alleles. Two alleles were observed in this study. Other studies have shown 6-PGD to have a dimeric structure (Vorsa et al.,1988; Hyun et al., 1987; Tsumura et al., 1989).

*Linkage studies* Loose linkage for *Aco*-1--*Pgm*-2--*Pgm*-1 was found and the recombination frequencies from pooled data were 37.21$\pm$4.39 percent and 33.30$\pm$5.82 percent, respectively (Fig. 4-2 and Table 4-6). 6-*Pgd*-2 was found to be tightly linked with the red leaf color locus in 3 crosses, and the recombination frequency was 0.00$\pm$0.06 percent (Table 4-6).

## Discussion

Five simply inherited traits and one linkage group have been described in hazelnut (Thompson et al., 1992). In this paper, nine additional loci and two linkage groups are identified. As more isozyme loci are identified, our knowledge of hazelnut genetics will increase.

Self-incompatibility, an inherited ability of a flower to reject its own pollen, effectively promotes outcrossing. Several isozyme loci have been found linked to the genes controlling gametophytic self-incompatibility (Tanksley and Loaiza-Figuera, 1985; Wricke and Wehling, 1985; Manganaris and Alston, 1987; Gertz and Wricke, 1989), but no isozyme loci have been reported linked to sporophytic self-incompatibility genes. Thompson (1985) found that pigmentation genes were linked to the sporophytic self-incompatibility S-locus in hazelnut. In our studies, we found 6-*Pgd-2* to be tightly linked to the red pigmentation gene in three crosses. No recombination between 6-*Pgd-2* and the red pigmentation gene was detected, but assortment was normal at the 6-Pgd-2 locus. Therefore, 6-*Pgd*-2 could serve as a biochemical marker in self-incompatibility studies in hazelnut.

Null alleles were found at *Pgm*-1 and *Pgm*-2, two loci which are linked with a recombination frequency of $33.30 \pm 5.82$ percent. Null alleles have been found at many isozyme loci and different crops, such as knobcone pine (Strauss and Conkle, 1986), almond (Hauagge et al.,1987), and apple (Manganaris and Alston, 1988b).

Table 4-1. Single locus segregation and goodness-of-fit for *Aco-1* and *Aco-2*.

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| | *Aco*-1 | | | | |
| 17-75 x Redleaf #3 | bc x cc | 25bc:30cc | 1:1 | 0.45 | 0.54 |
| 233-7 x 246-128 | bc x cc | 11bc:8cc | 1:1 | 0.47 | 0.49 |
| Tonda di Giffoni x Segorbe | bc x cc | 8bc:13cc | 1:1 | 1.20 | 0.27 |
| Casina x Redleaf #3 | bc x cc | 8bc:14cc | 1:1 | 1.60 | 0.20 |
| 30-13 x Rode Zeller | cc x bc | 13bc:10cc | 1:1 | 0.41 | 0.53 |
| USOR26-82 x Casina | cc x bc | 12bc:17cc | 1:1 | 0.87 | 0.35 |
| USOR14-82 x Casina | cc x bc | 9bc:10cc | 1:1 | 0.04 | 0.84 |
| Tombul x Tonda di Giffoni | cc x bc | 16bc:7cc | 1:1 | 3.62 | 0.06 |

Table 4-1. Single locus segregation and goodness-of-fit for *Aco*-1 and *Aco*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Fusco Rubra x Romisondo G-1 | cc x bc | 12bc:12cc | 1:1 | 1.00 | 0 |
| Tonda Romana x 23-17 | cc x bc | 16bc:10cc | 1:1 | 1.40 | 0.24 |
| Tonda Romana x 23-24 | cc x bc | 17bc:14cc | 1:1 | 0.29 | 0.59 |
| G pooled | | 147bc:145cc | 1:1 | 0.01 | 0.92 |
| G heterogeneity | | | | 10.3 | 0.41 |
| | | | | | |
| Fusco Rubra x 55-129 | cc x ac | 12ac:11cc | 1:1 | 0.04 | 0.84 |
| Tonda Romana x TGDL[2] | cc x ac | 13ac:12cc | 1:1 | 0.04 | 0.84 |
| 54-39 x TGDL | cc x ac | 11ac:12cc | 1:1 | 0.04 | 0.84 |

Table 4-1. Single locus segregation and goodness-of-fit for *Aco*-1 and *Aco*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G pooled | | 36ac:35cc | 1:1 | 0.01 | 0.92 |
| G heterogeneity | | | | 0.11 | 0.95 |
| Negret x Tonda di Giffoni | bc x bc | 21bb:55bc:20cc | 1:2:1 | 2.08 | 0.36 |
| Waterloo x 226-118 | bc x bc | 20bb:19bc:9cc | 1:2:1 | 6.38 | 0.04* |
| Tonda di Giffoni x GH184-19 | bc x bc | 4bb:7bc:6cc | 1:2:1 | 0.99 | 0.63 |
| G pooled | | 45bb:81bc:35cc | 1:2:1 | 1.26 | 0.26 |
| G heterogeneity | | | | 8.82 | 0.07 |

Table 4-1. Single locus segregation and goodness-of-fit for *Aco*-1 and *Aco*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tonda di Giffoni x 55-77 | bc x ac | 6ab:9ac:4bc:2cc | 1:1:1:1 | 5.30 | 0.15 |
| Sant Jaume x 55-77 | bc x ac | 6ab:6ac:0bc:10cc | 1:1:1:1 | 13.7 | .003** |
| 23-17 x TGDL | bc x ac | 7ab:13ac:25bc:23cc | 1:1:1:1 | 13.70 | .003** |
| G pooled | | 19ab:28ac:29bc:35cc | 1:1:1:1 | 4.90 | 0.18 |
| G heterogeneity | | | | 28.1 | .001** |
| | | | | | |
| 255-3 x Tonda di Giffoni | bb x bc | 58bb:42bc | 1:1 | 2.57 | 0.11 |
| Willamette x 23-17 | bb x bc | 12bb:21bc | 1:1 | 2.49 | 0.12 |
| G pooled | | 70bb:63bc | 1:1 | 0.37 | 0.54 |

Table 4-1. Single locus segregation and goodness-of-fit for *Aco*-1 and *Aco*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G heterogeneity | | | | 4.69 | 0.03* |
| Tombul Ghiaghli x Hall's Giant | cc x bb | 36bc | --- | --- | --- |
| TGDL x Hall's Giant | ac x bb | 4ab:5bc | 1:1 | 0.11 | 0.74 |
| Willamette x self | bb x bb | 23bb | --- | --- | --- |
| | *Aco*-2 | | | | |
| 43-91 x Imperial de Trebizonde | bb x bc | 23bb:25bc | 1:1 | 0.08 | 0.77 |
| Fusco Rubra x Romisondo G-1 | bb x bc | 12bb:12bc | 1:1 | 0 | 1.00 |

Table 4-1. Single locus segregation and goodness-of-fit for *Aco*-1 and *Aco*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Romai x 54-39 | bc x bb | 10bb:11bc | 1:1 | 0.05 | 0.81 |
| G pooled | | 45bb:48bc | 1:1 | 0.09 | 0.77 |
| G heterogeneity | | | | 0.04 | 0.98 |
| | | | | | |
| Tonda Romana x TGDL | ab x bb | 13ab:12bb | 1:1 | 0.04 | 0.82 |
| Tonda Romana x 244-1 | ab x bb | 11ab:9bb | 1:1 | 0.20 | 0.65 |
| Tonda Romana x 23-17 | ab x bb | 10ab:16bb | 1:1 | 1.40 | 0.24 |
| Tonda Romana x 23-24 | ab x bb | 17ab:14bb | 1:1 | 0.29 | 0.59 |
| G pooled | | 51ab:51bb | 1:1 | 0 | 1 |

Table 4-1. Single locus segregation and goodness-of-fit for *Aco*-1 and *Aco*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G heterogeneity | | | | 1.93 | 0.59 |
| Pendula x Aurea | bc x bc | 3bb:2bc:2cc | 1:2:1 | 1.56 | 0.46 |
| Fusco Rubra x Segorbe | bb x bb | 22bb | --- | --- | --- |

[z]Tonda Gentile delle Langhe

Table 4-2. Single locus segregation and goodness-of-fit for *Aap*-1, *Aap*-2, and *Aap*-3.

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| | *Aap*-1 | | | | |
| 233-7 x Daviana | bc x ac | 5ab:5ac:5bc:8cc | 1:1:1:1 | 1.08 | 0.78 |
| Imperiale de Trebizonde x 43-91 | bc x bb | 20bb:28bc | 1:1 | 1.34 | 0.25 |
| 296-82 x VR 8-32 | bb x ab | 11ab:9bb | 1:1 | 0.20 | 0.65 |
| 17-75 x Redleaf #3 | bb x bb | 106bb | --- | --- | --- |
| | *Aap*-2 | | | | |
| Waterloo x 226-118 | ab x bb | 32ab:16bb | 1:1 | 5.44 | 0.02* |
| Segorbe x Fusco Rubra | bb x ab | 11ab:13bb | 1:1 | 0.17 | 0.68 |

Table 4-2. Single locus segregation and goodness-of-fit for *Aap*-1, *Aap*-2, and *Aap*-3 (Contd.)

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G pooled | | 43ab:29bb | 1:1 | 2.74 | 0.10 |
| G heterogeneity | | | | 2.84 | 0.09 |
| 17-75 x Redleaf #3 | bb x bb | 106bb | --- | --- | --- |
| | *Aap*-3 | | | | |
| USOR 14-28 x Casina | bd x cd | 7bc:4bd:6cd:7dd | 1:1:1:1 | 0.99 | 0.81 |
| 23-17 x TGDL[z] | bd x cd | 9bc:24bd:13cd:27dd | 1:1:1:1 | 12.74 | 0.006** |
| TGDL x Hall's Giant | cd x bd | 2bc:2bd:2cd:3dd | 1:1:1:1 | 0.33 | 0.95 |

Table 4-2. Single locus segregation and goodness-of-fit for *Aap*-1, *Aap*-2, and *Aap*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tombul Ghiaghli x Hall's Giant | cd x bd | 9bc:9bd:8cd:10dd | 1:1:1:1 | 0.22 | 0.98 |
| Tonda Romana x 23-24 | cd x bd | 7bc:7bd:11cd:7dd | 1:1:1:1 | 4.34 | 0.23 |
| G pooled | | 34bc:46bd:40cd:54dd | 1:1:1:1 | 5.01 | 0.17 |
| G heterogeneity | | | | 13.61 | 0.32 |
| Redleaf #3 x 17-75 | cd x cd | 26cc:51cd:29dd | 1:1:1 | 0.62 | 0.73 |
| Casina x Redleaf #3 | cd x cd | 5cc:24cd:15dd | 1:2:1 | 5.60 | 0.06 |
| Tonda Romana x TGDL | cd x cd | 3cc:15cd:8dd | 1:2:1 | 2.98 | 0.23 |
| G pooled | | 34cc:90cd:52dd | 1:2:1 | 3.87 | 0.14 |

Table 4-2. Single locus segregation and goodness-of-fit for *Aap*-1, *Aap*-2, and *Aap*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G heterogeneity | | | | 5.53 | 0.24 |
| 225-77 X Hend 5-5 | bd x bd | 3bb:11bd:6dd | 1:1:1 | 1.22 | 0.27 |
| Waterloo x 226-118 | bd x bd | 10bb:31bd:9dd | 1:1:1 | 2.15 | 0.34 |
| G pooled | | 13bb:42:15dd | 1:1:1 | 2.98 | 0.23 |
| G heterogeneity | | | | 0.39 | 0.82 |
| Brixnut x Segorbe | bc x cd | 3bc:3bd:2cc:3cd | 1:1:1:1 | 0.26 | 0.98 |
| 233-7 x Daviana | cd x bc | 3bc:7bd:4cc:9cd | 1:1:1:1 | 3.96 | 0.27 |

Table 4-2. Single locus segregation and goodness-of-fit for *Aap*-1, *Aap*-2, and *Aap*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G pooled | | 6bc:10bd:6cc:12cd | 1:1:1:1 | 3.18 | 0.36 |
| G heterogeneity | | | | 1.04 | 0.79 |
| Negret x Tonda di Giffoni | dd x ac | 36ad:60cd | 1:1 | 6.00 | 0.02* |
| Tonda di Giffoni x 243-2 | ac x dd | 5ad:13cd | 1:1 | 3.68 | 0.56 |
| G pooled | | 41ad:73cd | 1:1 | 9.10 | 0.003** |
| G heterogeneity | | | | 0.58 | 0.45 |

Table 4-2. Single locus segregation and goodness-of-fit for *Aap*-1, *Aap*-2, and *Aap*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Sant Jaume x 55-77 | dd x bd | 15bd:11dd | 1:1 | 0.62 | 0.43 |
| Willamette x 23-17 | dd x bd | 16bd:10dd | 1:1 | 1.40 | 0.24 |
| G pooled | | 31bd:21dd | 1:1 | 1.93 | 0.16 |
| G heterogeneity | | | | 0.09 | 0.76 |
| | | | | | |
| Tonda di Giffoni x Segorbe | ac x cd | 6ac:3ad:6cc:5cd | 1:1:1:1 | 1.20 | 0.75 |
| 255-3 x Tonda di Giffoni | cd x ac | 26ac:27ad:20cc:27cd | 1:1:1:1 | 1.36 | 0.71 |
| G pooled | | 32ac:30ad:26cc:32cd | 1:1:1:1 | 0.83 | 0.84 |
| G heterogeneity | | | | 1.73 | 0.63 |

Table 4-2. Single locus segregation and goodness-of-fit for *Aap*-1, *Aap*-2, and *Aap*-3 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tombul x Tonda di Giffoni | bb x ac | 9ab:13bc | 1:1 | 0.93 | 0.39 |
| Segorbe x Fusco Rubra | cd x dd | 8cd:12dd | 1:1 | 0.80 | 0.38 |
| Pendula x Pellicle Rouge | dd x bc | 5bd:10cd | 1:1 | 1.67 | 0.19 |
| Tonda di Giffoni x GH184-19 | ac x ad | 1aa:4ac:8ad:4cd | 1:1:1:1 | 6.76 | 0.08 |
| 43-91 x Imperiale de Trebizonde | bc x bd | 5bb:7bc:14bd:21cd | 1:1:1:1 | 13.51 | 0.004** |
| Willamette x self | dd x dd | 23dd | ---- | | |

[z]Tonda Gentile delle Langhe

Table 4-3. Single locus segregation and goodness-of-fit for *Pgm*-1 and *Pgm*-2.

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| | *Pgm*-1 | | | | |
| USOR 14-28 x Casina | aa x ab | 11aa:11ab | 1:1 | 0 | 1.00 |
| 17-75 x Redleaf #3 | aa x ab | 60aa:35ab | 1:1 | 6.58 | 0.012* |
| 162-17 x Hend 5-5 | ab x aa | 7aa:13ab | 1:1 | 1.80 | 0.18 |
| G pooled | | 78aa:59ab | 1:1 | 2.64 | 0.10 |
| G heterogeneity | | | | 5.74 | 0.06 |
| | | | | | |
| Imperiale de Trebizonde x 43-91 | ab x bb | 13ab:10bb | 1:1 | 0.39 | 0.53 |
| USOR 24-82 x Purple Aveline | bb x ab | 20ab:13bb | 1:1 | 1.50 | 0.22 |

Table 4-3. Single locus segregation and goodness-of-fit for *Pgm*-1 and *Pgm*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Fusco Rubra x 55-129 | bb x ab | 10ab:12bb | 1:1 | 0.18 | 0.68 |
| Contorta x Redleaf #3 | bb x ab | 8ab:2bb | 1:1 | 3.92 | 0.06 |
| Fusco Rubra x Casina | bb x ab | 12ab:9bb | 1:1 | 0.39 | 0.53 |
| Waterloo x 226-118 | bb x ab | 21ab:27bb | 1:1 | 0.75 | 0.39 |
| G pooled | | 84ab:73bb | 1:1 | 0.77 | 0.38 |
| G heterogeneity | | | | 6.36 | 0.27 |
| TGDL[z] x 54-39 | ab x ab | 3aa:10ab:9bb | 1:2:1 | 4.37 | 0.11 |
| Romai x 54-39 | ab x ab | 6aa:8ab:7bb | 1:2:1 | 1.28 | 0.53 |

Table 4-3. Single locus segregation and goodness-of-fit for *Pgm*-1 and *Pgm*-2 (Contd.) .

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 255-3 x Tonda di Giffoni | ab x ab | 29aa:27ab:20bb | 1:2:1 | 8.83 | 0.01* |
| Casina x Readleaf #3 | ab x ab | 7aa:24ab:13bb | 1:2:1 | 2.19 | 0.33 |
| Willamette x self | ab x ab | 5aa:13ab:5bb | 1:2:1 | 0.40 | 0.82 |
| G pooled | | 50aa:82ab:54bb | 1:2:1 | 2.76 | 0.25 |
| G heterogeneity | | | | 14.3 | 0.07 |
| Pendula x Pellicle Rouge | bb x nn | 15bn | -- | --- | --- |
| 233-7 x Daviana | aa x nn | 23an | --- | --- | --- |
| Pendula x Aurea | bb x bb | 7bb | -- | --- | --- |

Table 4-3. Single locus segregation and goodness-of-fit for *Pgm*-1 and *Pgm*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Fusco Rubra x Segorbe | bb x aa | 24ab | --- | --- | --- |
| | *Pgm*-2 | | | | |
| Segorbe x Fusco Rubra | ac x cc | 12ac:8cc | 1:1 | 0.80 | 0.37 |
| USOR 24-82 x Purple Aveline | cc x ac | 19ac:14cc | 1:1 | 0.76 | 0.38 |
| Fusco Rubra x 55-129 | cc x ac | 8ac:12cc | 1:1 | 0.80 | 0.37 |
| Tombul x Tonda di Giffoni | cc x ac | 13ac:10cc | 1:1 | 0.39 | 0.53 |
| G pooled | | 52ac:44cc | 1:1 | 0.66 | 0.42 |
| G heterogeneity | | | | 0.09 | 0.55 |

Table 4-3. Single locus segregation and goodness-of-fit for *Pgm*-1 and *Pgm*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 255-3 x Tonda di Giffoni | aa x ac | 33aa:43ac | 1:1 | 1.62 | 0.20 |
| TGDL x 54-39 | ac x aa | 8aa:15ac | 1:1 | 2.12 | 0.14 |
| G pooled | | 41aa:58ac | 1:1 | 6.93 | 0.008* |
| G Heterogeneity | | | | 3.19 | 0.07 |
| Pendula x Pellicle Rouge | aa x ab | 7aa:8ab | 1:1 | 0.06 | 0.80 |
| Romai x 54-39 | ab x aa | 11aa:10ab | 1:1 | 0.05 | 0.83 |
| G pooled | | 18aa:18ab | 1:1 | 1.00 | 0 |
| G heterogeneity | | | | 0.89 | 0.35 |

Table 4-3. Single locus segregation and goodness-of-fit for *Pgm*-1 and *Pgm*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 233-7 x Daviana | cc x aa | 23ac | --- | --- | --- |
| Contorta x Redleaf #3 | aa x nn | 10an | --- | --- | --- |
| Pendula x Aurea | aa x nn | 7an | --- | --- | --- |
| Casina x Redleaf #3 | nn x nn | 44nn | --- | --- | --- |

[z]Tonda Gentile delle Langhe

Table 4-4. Single locus segregation and goodness-of-fit for *Sdh*-2

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Fusco Rubra x Romisondo G-1 | aa x ab | 12aa:9ab | 1:1 | 0.43 | 0.51 |
| Sant Jaume x 55-77 | aa x ab | 12aa:14ab | 1:1 | 0.15 | 0.70 |
| Fusco Rubra x Romisondo G-1 | aa x ab | 9aa:15ab | 1:1 | 1.50 | 0.22 |
| Willamette x 23-17 | aa x ab | 15aa:21ab | 1:1 | 1.01 | 0.32 |
| USOR 24-82 x Casina | ab x aa | 9aa:12ab | 1:1 | 0.43 | 0.51 |
| USOR 26-28 x Casina | ab x aa | 14aa:19ab | 1:1 | 0.76 | 0.38 |
| 162-17 x Hend 5-5 | ab x aa | 6aa:14ab | 1:1 | 3.29 | 0.07 |
| 296-82 x VR 8-32 | ab x aa | 12aa:8ab | 1:1 | 0.80 | 0.37 |
| Tombul x Tonda di Giffoni | ab x aa | 10aa:13ab | 1:1 | 0.39 | 0.53 |
| Pendula x Aurea | ab x aa | 5aa:3ab | 1:1 | 0.13 | 0.72 |

Table 4-4. Single locus segregation and goodness-of-fit for *Sdh*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tombul Ghiaghli x Hall's Giant | ab x aa | 14aa:22ab | 1:1 | 1.78 | 0.18 |
| 243-2 x Tonda di Giffoni | ab x aa | 9aa:9ab | 1:1 | 0 | 1.00 |
| Waterloo x 226-118 | ab x aa | 28aa:20ab | 1:1 | 1.30 | 0.25 |
| Tonda Romana x 244-1 | ab x aa | 9aa:11ab | 1:1 | 0.20 | 0.65 |
| Tonda Romana x 23-24 | ab x aa | 15aa:16ab | 1:1 | 0.03 | 0.86 |
| G pooled | | 179aa:206ab | 1:1 | 1.91 | 0.17 |
| G heterogeneity | | | | 10.3 | 0.74 |
| TGDL[z] x Gem | ad x aa | 11aa:12ad | 1:1 | 0.04 | 0.84 |
| TGDL x Mortarella | ad x aa | 9aa:14ad | 1:1 | 1.09 | 0.30 |

Table 4-4. Single locus segregation and goodness-of-fit for *Sdh*-2 (Contd.)

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| TGDL x Hall's Giant | ad x aa | 3aa:6ad | 1:1 | 1.00 | 0.32 |
| G pooled | | 23aa:32ad | 1:1 | 1.48 | 0.22 |
| G heterogeneity | | | | 0.65 | 0.72 |
| Tonda Romana x TGDL | ab x ad | 3aa:9ab:8ad:5bd | 1:1:1:1 | 3.87 | 0.26 |
| TGDL x 23-17 | ad x ab | 18aa:12ab:21ad:20bd | 1:1:1:1 | 2.87 | 0.41 |
| G pooled | | 21aa:21ab:29ad:25bd | 1:1:1:1 | 1.80 | 0.62 |
| G heterogeneity | | | | 4.94 | 0.18 |

Table 4-4. Single locus segregation and goodness-of-fit for *Sdh*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 55-129 x 243-2 | bd x ab | 3ab:4ad:6bb:7bd | 1:1:1:1 | 2.00 | 0.58 |
| USOR24-82 x Purple Aveline | ab x bc | 12ab:5ac:2bb:10bc | 1:1:1:1 | 9.65 | 0.03* |
| Pellicle Rouge x Pendula | ab x ab | 2aa:9ab:4bb | 1:2:1 | 1.13 | 0.53 |
| Tonda Romana x 23-17 | ab x ab | 10aa:10ab:6bb | 1:2:1 | 2.41 | 0.30 |
| G pooled | | 12aa:19ab:10bb | 1:2:1 | 0.41 | 0.81 |
| G heterogeneity | | | | 3.13 | 0.21 |
| 39-44 x Imperiale de Trebizonde | aa x ac | 12aa:12ac | 1:1 | 0 | 1.00 |
| Imperiale de Trebizonde x 43-91 | ac x aa | 23aa:25ac | 1:1 | 0.08 | 0.77 |

Table 4-4. Single locus segregation and goodness-of-fit for *Sdh*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| G pooled | | 35aa:37ac | 1:1 | 0.07 | 0.79 |
| G heterogeneity | | | | 0.01 | 0.92 |
| Fusco Rubra x 55-129 | aa x bd | 12ab:11ad | 1:1 | 0.04 | 0.84 |

[z]Tonda Gentile delle Langhe

Table 4-5. Single locus segregation and goodness-of-fit for 6-*Pgd*-2.

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Negret x Tonda di Giffoni | aa x ab | 54aa:42ab | 1:1 | 1.50 | 0.22 |
| 255-3 x Tonda di Giffoni | aa x ab | 57aa:43ab | 1:1 | 2.57 | 0.11 |
| Tonda di Giffoni x 55-77 | ab x aa | 12aa:11ab | 1:1 | 0.04 | 0.84 |
| USOR 24-82 x Purple Aveline | ab x aa | 18aa:15ab | 1:1 | 0.27 | 0.60 |
| USOR 26-82 x Casina | ab x aa | 17aa:16ab | 1:1 | 0.03 | 0.86 |
| G pooled | | 158aa:127ab | 1:1 | 3.37 | 0.07 |
| G heterogeneity | | | | 1.40 | 0.84 |
| Ribet x Segorbe | ab x ab | 8aa:13ab:7bb | 1:2:1 | 0.16 | 0.93 |

Table 4-5. Single locus segregation and goodness-of-fit for 6-*Pgd*-2 (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| 30-13 x Rode Zeller | bb x ab | 9ab:14bb | 1:1 | 1.08 | 0.30 |
| Fusco Rubra x Gem | aa x aa | 23aa | ---- | --- | --- |
| Fusco Rubra x 55-129 | aa x aa | 22aa | --- | ---- | --- |

Table 4-6. Linkage and recombination frequencies for five loci in hazelnut.

| Locus pair | Cross | Number of progeny | G | P | Recombination value ± SE |
|---|---|---|---|---|---|
| *Aco*-1/*Pgm*-1 | Waterloo x 226-118 | 48 | 4.42 | 0.05 | 60.71 ± 10.07 |
| | 255-3 x Tonda di Giffoni | 100 | 6.40 | 0.04 | 55.06 ± 8.12 |
| | Romai x 54-39 | 21 | 10.33 | 0.01 | 24.39 ± 8.07 |
| | Pooled | 169 | 6.20 | 0.05 | 53.23 ± 7.13 |
| *Aco*-1/*Pgm*-2 | 54-39 x TGDL | 23 | 4.25 | 0.04 | 48.17 ± 9.93 |
| | 255-3 x Tonda di Giffoni | 100 | 6.23 | 0.02 | 28.98 ± 5.73 |
| | Romai x 54-39 | 21 | 3.85 | 0.05 | 45.99 ± 12.65 |
| | Pooled | 144 | 8.01 | 0.00 | 37.21 ± 4.39 |
| *Pgm*-1/*Pgm*-2 | 54-39 x TGDL[z] | 23 | 12.80 | 0.00 | 16.65 ± 11.23 |
| | 255-3 x Tonda di Giffoni | 100 | 38.40 | 0.00 | 36.70 ± 7.87 |

Table 4-6. Linkage and recombination frequencies for four loci in hazelnut (Contd.).

| Locus pair | Cross | Number of progeny | G | P | Recombination value ± SE |
|---|---|---|---|---|---|
| | Romai x 54-39 | 21 | 14.35 | .0002 | 15.37 ± 11.40 |
| | USOR 24-82 x Purple Aveline | 33 | 3.89 | 0.05 | 33.32 ± 8.22 |
| | Pooled | 177 | 40.00 | 0.00 | 33.30 ± 5.82 |
| 6*Pgd*-2/Red leaf | USOR 26-82 x Casina | 33 | 31.73 | 0.00 | 0.00 ± 0.06 |
| | USOR 24-82 x Purple Aveline | 33 | 43.86 | 0.00 | 0.00 ± 0.06 |
| | 30-13 x Rode Zeller | 23 | 30.79 | 0.00 | 0.00 ± 0.07 |

[z]Tonda Gentile delle Langhe

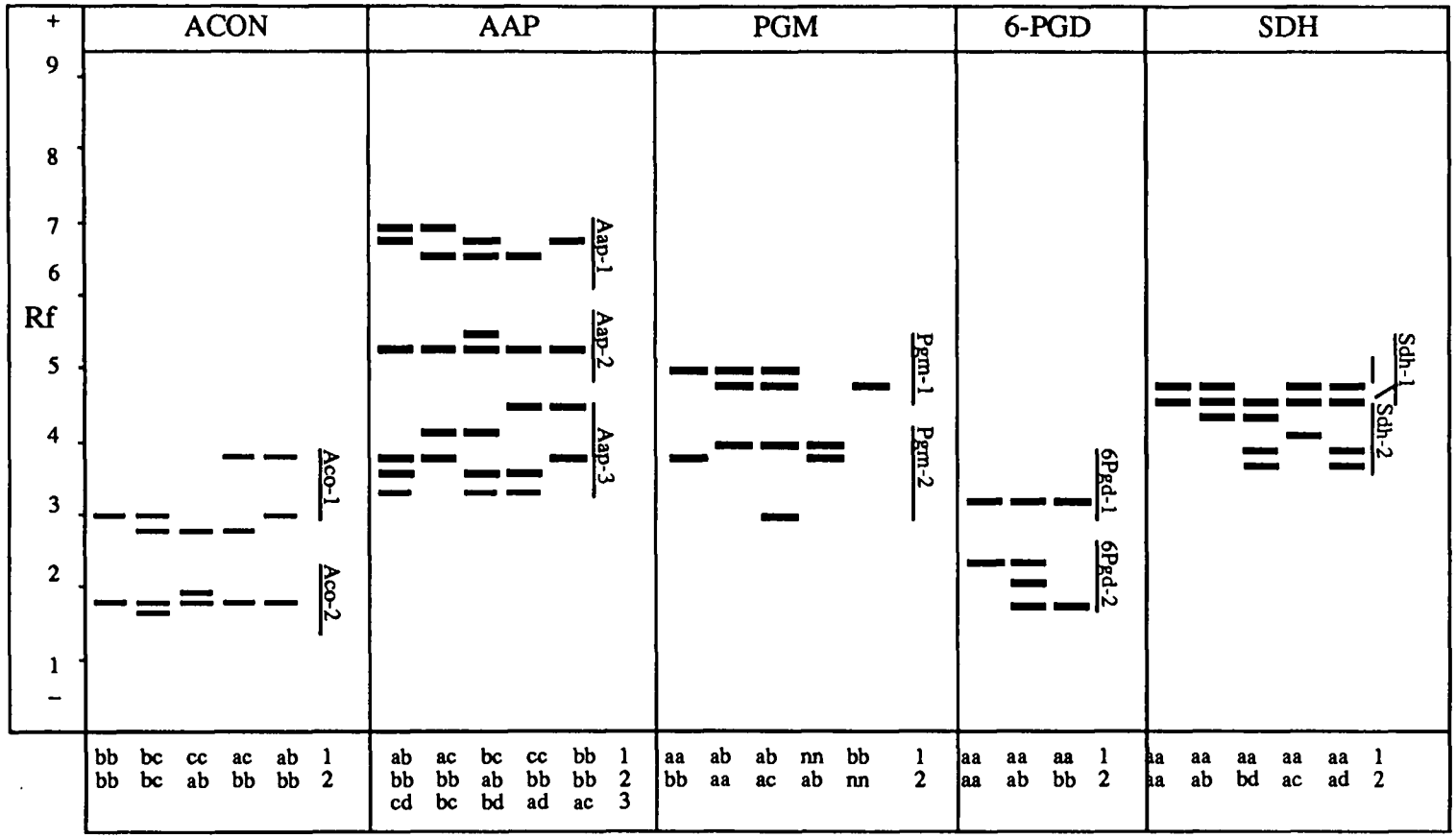


Fig. 4-1. Schematic presentation of allelic variation of ACON, AAP, PGM, 6-PGD, and SDH in hazelnut.

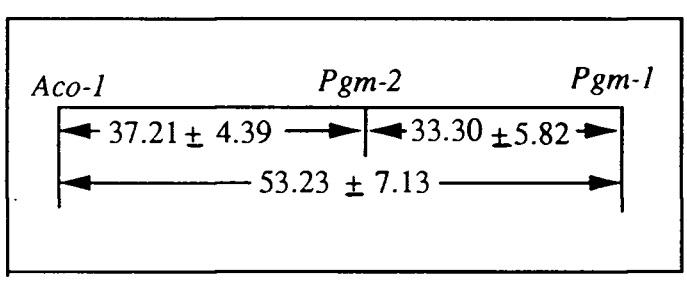


Fig. 4-2. Isozyme linkage map for Aco-1, Pgm-2 and Pgm-1.

## Literature Cited

Arulsekar, S., D. E. Parfitt, W. Beres, and P. E. Hansche. 1986. Genetics of malate dehydrogenase isozymes in the peach. J. Hered. 77:49-51.

Arulsekar, S. and D. E. Parfitt. 1986. Isozyme analysis procedures for stone fruit, almond, grape, walnut, pistachio, and fig. HortScience 21:928-933.

Cardy, B. J., C. W. Stuber, and M. M. Goodman. 1980. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Inst. Stat. Mimeo. Ser. 1317, North Carolina State Univ., Raleigh.

Cheng, S. Z. and W. H. Yang. 1987. Taxonomic studies of ten species of the genus *Juglans* based on isozymic zymograms. Acta Hort. Sinica 14:90-96.

Chevreau, E., Y. Lespinasse, and M. Gallet. 1985. Inheritance of pollen enzymes and polyploid origin of apple. Theor. Appl. Genet. 71:268-277.

Chevreau, E., and F. Laurens. 1987. The pattern of inheritance in apple: further results from leaf isozyme analysis. Theor. Appl. Genet. 75: 90-95.

Doong, J.-Y. H., and Y.-T Kiang. 1987. Inheritance of aconitase isozymes in soybean. Genome 29:713-717.

Durham, R. E., G. A. Moore, and W. B. Sherman. 1987. Isozyme banding patterns and their usefulness as genetic markers in peach. J. Amer. Soc. Hort. Sci. 112:1013-1018.

Gertz, A. and G. Wricke. 1989. Linkage between the incompatibility locus Z and 1-glucosidase locus in rye. Plant Breeding 102:255-259.

Hauagge, R., D. E. Kester, and R. A. Asay. 1987. Isozyme variation among California almond cultivars : 1. Inheritance. J. Amer. Soc. Hort. Sci. 112: 687-693.

Hyun, J. O., O. J. Rajora, and L. Zsuffa. 1987. Inheritance and linkage of isozymes in *Populus tremuloides* (Michx.). Genome 29:384-388.

Liu, B. H. and S. J. Knapp. 1990. G-MENDEL: a program for Mendelian segregation and linkage analysis of individual or multiple progeny populations using log-likelihood ratios. J. Hered. 81:407.

Loukas, M., M. N. Stavrakasis, and C. B. Krimbas. 1983. Inheritance of polymorphic isozymes in grape cultivars. J. Hered. 74:181-183.

Manganaris, A. G., and F. H. Alston. 1987. Inheritance and linkage relations of GOT isozymes in apple: 1. the gene GOT-1, a marker for the S incompatibility locus. Theor. Appl. Genet. 74:154-161.

Manganaris, A. G., and F. H. Alston. 1988a. Inheritance and linkage relations of GOT isozymes in apple: 2. The genes GOT-2 and GOT-4. Theor. Appl. Genet. 76:449-454.

Manganaris, A. G. and F. H. Alston. 1988b. The acid phosphatase gene *Acp-1* and its linkage with the endopeptidase gene *Enp-1* and the pale green lethal gene 1 in apple. Acta Hort. 224:177-184.

Mowrey, B. D., D. J. Werner, and D. H. Byrne. 1990. Inheritance of

isocitrate dehydrogenase, malate dehydrogenase, and shikimate dehydrogenase in peach and peach x almond hybrids. J. Amer. Soc. Hort. Sci. 115:312-319.

Parfitt, D. E. and S. Arulsekar. 1989. Inheritance and isozyme diversity for GPI and PGM among grape cultivars. J. Amer. Soc. Hort. Sci. 114:486-491.

Pitel, J. A., W. M. Cheliak, and J. Barrett. 1987. Inheritance of allozymes in a black spruce diallele cross. Silvae Genet. 36:149-153.

Strauss, S. H. and M. T. Conkle. 1986. Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. 72:483-493.

Tanksley, S. D. and Loaiza-Figuera F. 1985. Gametophytic self-incompatibility is controlled by a single major locus on chromosome 1 in *Lycopersicon peruvianum*. Proc. Natl. Acad. Sci. 82:5093-5096.

Thompson, M. M. 1985. Linkage of the incompatibility locus and red pigmentation genes in hazelnut. J. Hered. 76:119-122.

Thompson, M. M., H. B. Lagerstedt, and S. A. Mehlenbacher. 1992. Hazelnuts. In: J. Janick and J. N. Moore (eds.), Advances in Fruit Breeding, Vol. 2. (in press).

Tsumura, Y., K. Uchida, and K. Ohba. 1989. Genetic control of isozyme variation in needle tissues of *Cryptomeria japonica*. J. Hered. 80:291-297.

van Heemstra, M.I., L. P. Bruederle, and N. Vorsa. 1991. Inheritance and

linkage of thirteen polymorphic isozyme loci in diploid blueberry. J. Amer. Soc. Hort. Sci. 116:89-94.

Vorsa, N., P. S. Manos, and van Heemstra, M. I. 1988. Isozyme variation and inheritance in blueberry. Genome 30:776-781.

Weeden, N. F. and R. C. Lamb. 1987. Genetics and linkage analysis of 19 isozyme loci in apple. J. Amer. Soc. Hort. Sci. 112:865-872.

Weeden, N. F., B. I. Reisch, and Mary-Howell E. Maartens. 1988. Genetic analysis of isozyme polymorphism in grape. J. Amer. Soc. Hort. Sci. 113: 765-769.

Wricke, G. and P. Wehling. 1985. Linkage between an incompatibility locus and a peroxidase isozyme locus in rye. Theor. Appl. Genet. 71:289-291.

Chapter 5

# IDENTIFYING HAZELNUT CULTIVARS BY ISOZYME ANALYSIS

## Abstract

Eight enzyme systems were resolved in hazelnut (*Corylus avellana* L.) by either polyacrylamide gel electrophoresis (PAGE) or starch gel electrophoresis. Of the 17 isozymic loci studied, 13 were polymorphic. The most useful enzyme systems for hazelnut cultivar identification were alanine aminopeptidase (AAP), phosphoglucomutase (PGM), aconitase (ACON), and glucose phosphate isomerase (GPI). 54 of 56 cultivars studied could be distinguished by a combination of the isozymes examined. Thus, isozyme analysis is a very powerful tool for identifying hazelnut cultivars. 'Tombul' and 'Extra Ghiaghli' appear to be the same cultivar based on both isozyme analysis and morphological characteristics.

## Introduction

Most important hazelnut cultivars are from *Corylus avellana* L., which is native to Europe and Asia Minor and rich in genetic diversity (Mehlenbacher, 1990). Detailed descriptions are not available for most cultivars. Traditional identification is usually based on morphological traits, such as plant size and growth habit, nut size, nut shape, and husk length, which require bearing trees and can be influenced by crop load and environmental conditions. Clonal cultivars grown in many countries frequently have more than one name. In other cases, groups of similar clones are called the same name but are genetically distinct. A reliable and simple method is needed for identifying hazelnut cultivars using young trees.

Isozymes have been employed widely in identifying cultivars, determining phylogenetic relationships within and among species, and confirming parentage in fruit and nut crops, including almond (Arulsekar et al., 1986; Hauagge et al., 1987; Cerezo et al., 1989), apple (Menendez et al., 1984; Weeden and Lamb, 1985), apricot (Byrne and Littleton, 1989), grape (Wolfe, 1976; Chaparro et al., 1989), kiwifruit (Messina et al, 1991), persimmon (Tao and Sugiura, 1987), plum (Parfitt et al., 1985; Byrne and Littleton, 1988), peach (Arulsekar et al., 1986b; Messeguer et al., 1987; Durham et al., 1988), pineapple (Dewald et al., 1988), mango (Degani et al., 1990), and walnut (Cheng and Yang, 1987). Advantages of using isozymes include the absence of significant physiological effects, the simple

genetic basis of most variation and the codominant expression displayed, the lack of epistatic or pleiotropic interactions among different loci, and expression that is generally independent of environmental conditions, rootstocks, and tissue sampled (Weeden, 1989).

Ahmad et al. (1987) extracted proteins from 1-year-old shoots of hazelnut and examined three polymorphic enzyme systems--peroxidase, phenol oxidase and acid phosphatase. Because young seedlings are damaged when shoots are collected during the growing season, we developed a method based on young leaves instead of shoots.

The objectives of our study were to characterize the world's important hazelnut cultivars and the parents used extensively in the Oregon State University (OSU) hazelnut breeding program using isozyme polymorphisms, and to identify possible duplication in the collection.

## Materials and Methods

The 56 cultivars used in this study originated from several different countries and were grown at both the OSU Vegetable Farm and the National Clonal Germplasm Repository (NCGR), Corvallis, Oregon (Table 1). Young leaves were collected during the growing seasons and carried to the lab in plastic bags for immediate use or stored at 4 C for up to 3 days. The extraction procedures, gel running, and staining procedures have been described elsewhere (Chapter 4 and 5).

## Results and Discussion

Fourteen enzyme systems were assayed by either polyacrylamide or starch gel electrophoresis. No enzyme activity was observed for alcohol dehydrogenase, amylase, diaphorase, glutamate dehydrogenase, and isocitrate dehydrogenase. In two enzyme systems, triose phosphate isomerase and menadione reductase, no variation was observed. Good and consistent results were obtained and differences among cultivars were observed for the eight enzymes described below.

*Alanine aminopeptidase* (AAP) Considerable variation among the cultivars was observed for AAP. The three zones of activity, all polymorphic, were designated *Aap*-1, *Aap*-2 and *Aap*-3 (Fig. 5-1) and all were found to be monomeric (Cheng et al., 1992a). Three alleles, a, b, and c, were detected at the *Aap*-1 locus. The b allele was most common and the a allele was only observed in 'Daviana' (Table 5-1).

Two alleles, designated a and b, were found at the *Aap*-2 locus. The b allele was by far the most common in the cultivars (Table 5-1). An additional band towards the anode was found in some interspecific hybrids.

*Aap*-3 showed great variation among the cultivars and was the most useful locus for cultivar identification (Table 5-1 and Fig. 5-1). Five alleles were identified; the c, b, and d alleles were the most common. The a allele appeared only in 'Riccia di Talanico' and 'Tonda di Giffoni' and the e allele only in 'Creswell', 'Italian Red', 'Neue Riesennuss', and 'Ugbrooke'. The cd phenotype

was relatively common among the cultivars. Based on the *Aap*-3 locus, the cultivars examined could be divided into 11 groups.

AAP is rarely investigated. In *Cryptomeria japonica*, only one locus with two alleles was found (Tsumura et al., 1989). The *Aap*-3 locus is inferred to have evolved more divergently than any other loci examined, or the genome coding for this locus has particularly diverse regions in hazelnut.

*Aspartate aminotransferase* (AAT) AAT had only one cathode area of staining (Fig. 5-2). Genetic analysis showed that AAT was dimeric with 2 alleles at a single locus (Chapter 3). Only two phenotypes were observed, single-banded (bb) and triple-banded patterns (ab). Among the 56 cultivars investigated, 46 had the bb phenotype (Table 5-1). No aa cultivars were observed, although such seedlings have been obtained from controlled crosses.

*Aconitase* (ACON) Two zones of enzyme activity were identified on starch gel slices stained for ACON (Fig 5-3). Both were monomeric and controlled by loci (*Aco*-1 and *Aco*-2) with 3 alleles each (Chapter 4). For *Aco*-1, the b and c alleles were common and the a allele was only found in 'Tonda Gentile delle Langhe'. When only one band appeared, it stained strongly. The cc and bc phenotypes were relatively common. The bb phenotype was observed in 'Gasaway', 'Willamette', 'Hall's Giant', and 'Italian Red'. The ac phenotype was observed only in 'Tonda Gentile delle Langhe'.

For *Aco*-2, the b allele was present in all cultivars; the c allele was present in 'Riccia di Talanico', 'Romisondo G-1', 'Imperiale de Trebizonde', 'Aurea', and

'Pendula' and the a allele was only found in 'Romai'. Three phenotypes, ab, bb, and bc, were observed. The bb phenotype appeared frequently (Table 5-1).

*Glucose phosphate isomerase* (GPI) Three GPI zones of activity were observed (Fig. 5-4). GPI-1 was often diffuse. *Gpi*-2 and *Gpi*-3 were polymorphic with 2 alleles each. *Gpi*-2 was dimeric and *Gpi*-3 was monomeric (Chapter 3). For Gpi-2, only two phenotypes, either single-banded (bb) or triple-banded (ab), were observed. The triple-banded pattern appeared only in three cultivars, 'Camponica', 'Tonda di Giffoni', and 'Segorbe'. No aa phenotype was found among the cultivars studied. For *Gpi*-3, the aa and ab phenotypes were relatively common (Table 5-1). The interlocus *Gpi*-x was observed only in 'Camponica', 'Tonda di Giffoni', and 'Segorbe'.

*6-Phosphogluconate dehydrogenase* (6-PGD) 6-PGD had two activity zones (Fig. 5-5). 6*Pgd*-1 was monomorphic and 6*Pgd*-2 was polymorphic and dimeric with three alleles (Chapter 4). The a allele was most common. There were four phenotypes for Pgd-2: aa, ab, ac, and bb. The aa phenotype appeared frequently (Table 5-1).

*Phosphoglucomutase* (PGM) PGM showed two areas of activity (Fig. 5-6). *Pgm*-1 and *Pgm*-2 were both polymorphic and monomeric (Chapter 4). *Pgm*-1 had three alleles: a, b, and n. The a and b alleles were relatively common. *Pgm*-2 had four alleles: a, b, c, and n. The a allele was most common (Table 5-1). *Pgm*-2 was useful for hazelnut cultivar identification. Both *Pgm*-1 and *Pgm*-2 had null alleles.

*Malate dehydrogenase* (MDH) MDH had three zones of activity (Fig. 5-7). MDH was a dimeric enzyme (Chapter 3)). *Mdh*-2 and *Mdh*-3 were monomorphic among the cultivars examined, but a triple-banded pattern was found in a few interspecific cultivars for *Mdh*-3. *Mdh*-1 was polymorphic. Only two phenotypes, one single-banded (bb) and the other triple-banded (ab), were found (Table 5-1). Of the 56 cultivars, 42 had the bb phenotype.

*Shikimate dehydrogenase* (SDH) Two isozymes were observed. *Sdh*-1 was monomorphic and *Sdh*-2 was polymorphic (Fig. 5-8). *Sdh*-2 was monomeric with four alleles (Chpater 4). The a allele was the most common and the c allele was found only in 'Imperial de Trebizonde'. The aa phenotype was the most common (Table 5-1).

Of the 17 isozyme loci studied, 13 were polymorphic. 54 of 56 cultivars could be distinguished by a combination of the isozymes studied. The most useful enzyme systems for hazelnut cultivar identification were AAP, followed by PGM, ACON, and GPI. Isozyme analysis is a powerful tool for identifying hazelnut cultivars.

Isozyme analysis proved useful in resolving a nomenclatural problem. 'Tombul' and 'Extra Ghiaghli' from Turkey and Greece, respectively, were collected as two different cultivars. These cultivars were identical with respect to morphological traits, including plant size and growth habit, nut size and shape, percent kernel, husk length, leaf size and shape, and susceptibility to big bud mite

(*Phytoptus avellanae* Nal.). Since analysis of 8 enzyme systems also could not distinguish these two cultivars, it appears highly likely that 'Tombul' and 'Extra Ghiaghli' are the same cultivar.

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars.

| Country | Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Aco1* | *Aco2* | *Aap1* | *Aap2* | *Aap3* | *Gpi2* | *Gpi3* | *Pgm1* | *Pgm2* | *Sdh2* | *Aat* | *Mdh1* | *Pgd2* |
| Italy | Camponica | bc | bb | bb | bb | cd | ab | aa[z] | aa | aa | aa | ab | bb | ac |
| | Montebello | bc | bb | bb | bb | cd | bb | aa | aa | aa | aa | bb | bb | aa |
| | Mortarella | bc | bb | bb | bb | dd | bb | ab | aa | aa | aa | bb | bb | ac |
| | Riccia di Talanico | bc | bc | bb | bb | ad | bb | aa | bb | aa | aa | ab | ab | aa |
| | Romisondo G-1 | bc | bc | bb | bb | cd | bb | aa | aa | aa | ab | bb | ab | ab |
| | Tonda di Giffoni | bc | bb | bb | bb | ac | ab | ab | ab | ac | aa | ab | ab | ab |
| | Tonda Gentile d. Langhe | ac | bb | bb | bb | cd | bb | aa | ab | ac | ad | bb | bb | aa |
| | Tonda Romana | cc | ab | bb | bb | cd | bb | aa | bb | aa | ab | bb | ab | aa |
| Spain | Barcelona | bc | bb | bb | bb | cd | bb | ab | aa | aa | aa | bb | bb | aa |
| | Casina | bc | bb | bb | bb | cd | bb | ab | ab | nn | aa | ab | ab | aa |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued).

| Country | Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Aco*1 | *Aco*2 | *Aap*1 | *Aap*2 | *Aap*3 | *Gpi*2 | *Gpi*3 | *Pgm*1 | *Pgm*2 | *Sdh*2 | *Aat* | *Mdh*1 | *Pgd*2 |
| | Gironell (=Grossal) | bc | bb | bb | bb | cd | bb | aa | nn | aa | aa | ab | bb | aa |
| | Negret | bc | bb | bb | bb | dd | bb | aa | aa | aa | aa | bb | ab | aa |
| | Pauetet | bc | bb | bb | bb | dd | bb | aa | aa | aa | aa | bb | bb | aa |
| | Ribet | cc | bb | bb | bb | cd | bb | aa | aa | aa | aa | bb | bb | ab |
| | Sant Jaume | bc | bb | bb | bb | dd | bb | aa | aa | aa | aa | bb | bb | aa |
| | Sant Joan | cc | bb | bb | bb | cd | bb | aa | bb | aa | aa | bb | ab | ab |
| | Sant Pere | bc | bb | bb | bb | cd | bb | aa | aa | aa | aa | bb | ab | aa |
| | Segorbe | cc | bb | bb | bb | cd | ab | aa | aa | ac | aa | bb | bb | ab |
| USA | Butler | cc | bb | bc | bb | cd | bb | aa | nn | aa | aa | bb | bb | ab |
| | Brixnut | cc | bb | bb | bb | bc | bb | ab | bb | bb | aa | bb | bb | aa |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued)

| Country / Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Aco1* | *Aco2* | *Aap1* | *Aap2* | *Aap3* | *Gpi2* | *Gpi3* | *Pgm1* | *Pgm2* | *Sdh2* | *Aat* | *Mdh1* | *Pgd2* |
| Creswell | cc | bb | bb | bb | ce | bb | aa | aa | cc | ad | bb | bb | aa |
| Ennis | bc | bb | bc | bb | bc | bb | ab | aa | aa | aa | bb | bb | ab |
| Gasaway | bb | bb | bc | bb | dd | bb | ab | bb | aa | ab | bb | bb | aa |
| Gem | bc | bb | bb | bb | bc | bb | aa | nn | aa | aa | bb | bb | aa |
| Nonpareil | bc | bb | bb | bb | cd | bb | ab | nn | aa | aa | bb | bb | ab |
| Nooksack | bc | bb | bb | bb | bb | bb | ab | bb | aa | aa | ab | bb | aa |
| Royal | cc | bb | bc | bb | bd | bb | ab | bb | aa | aa | bb | bb | ab |
| Ryan | cc | bb | bb | bb | bc | bb | ab | aa | aa | aa | bb | bb | ab |
| Willamette (OSU 43-58) | bb | bb | bb | bb | dd | bb | aa | ab | aa | aa | bb | bb | ab |
| Woodford | cc | bb | bc | bb | cd | bb | ab | aa | aa | aa | bb | bb | ab |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued).

| Country | Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Aco*1 | *Aco*2 | *Aap*1 | *Aap*2 | *Aap*3 | *Gpi*2 | *Gpi*3 | *Pgm*1 | *Pgm*2 | *Sdh*2 | *Aat* | *Mdh*1 | *Pgd*2 |
| Turkey | Badem | cc | bb | bb | bb | cd | bb | ab | aa | aa | ab | ab | bb | aa |
| | Imperiale de Trebizonde | cc | bc | bc | bb | bd | bb | bb | ab | nn | ac | bb | ab | aa |
| | Palaz | cc | bb | bb | bb | bd | bb | ab | aa | cc | ab | bb | bb | aa |
| | Sivri Ghiaghli | cc | bb | bb | bb | cd | bb | aa | nn | ac | ab | bb | bb | aa |
| | Tombul(=Extra Ghiaghli) | cc | bb | bb | bb | bb | bb | ab | aa | cc | ab | bb | bb | aa |
| | Tombul Ghiaghli | cc | bb | bb | bb | cd | bb | ab | ab | cc | ab | ab | bb | ab |
| Germany | Hall's Giant | bb | bb | bb | bb | bd | bb | aa | bb | aa | aa | bb | bb | aa |
| | Italian Red | bb | bb | bb | bb | de | bb | aa | bb | aa | ab | bb | bb | aa |
| | Neue Riesennuss | cc | bb | bb | bb | ce | bb | aa | bb | bb | aa | bb | bb | bb |
| England | Cosford | cc | bb | bc | bb | bd | bb | aa | bb | ab | aa | bb | bb | aa |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued).

| Country | Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Aco*1 | *Aco*2 | *Aap*1 | *Aap*2 | *Aap*3 | *Gpi*2 | *Gpi*3 | *Pgm*1 | *Pgm*2 | *Sdh*2 | *Aat* | *Mdh*1 | *Pgd*2 |
| | Duchilly(=Kentish Cob) | bc | bb | bb | bb | cd | bb | bb | nn | aa | ab | bb | bb | aa |
| | Daviana | cc | bb | ac | bb | bc | bb | ab | nn | aa | aa | bb | bb | bb |
| France | GN66(3)AF5 | bc | bb | bc | bb | bb | bb | aa | bb | nn | aa | bb | bb | ab |
| | Henneman #3 | cc | bb | bb | bb | cd | bb | ab | nn | bc | aa | bb | bb | aa |
| Other countries | | | | | | | | | | | | | | |
| | Pellicle Rouge | cc | bb | bb | bb | bc | bb | ab | nn | ab | ab | bb | ab | aa |
| | Romai | bc | bc | bb | bb | cd | bb | aa | ab | ab | aa | bb | ab | ac |
| | Ugbrooke | cc | bb | bc | bb | be | bb | ab | bb | cc | aa | bb | ab | aa |
| | Waterloo | bc | bb | bb | ab | bd | bb | aa | bb | aa | ab | ab | ab | aa |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued)

| Country / Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Aco1* | *Aco2* | *Aap1* | *Aap2* | *Aap3* | *Gpi2* | *Gpi3* | *Pgm1* | *Pgm2* | *Sdh2* | *Aat* | *Mdh1* | *Pgd2* |
| **Ornamentals** | | | | | | | | | | | | | |
| Aurea | cc | bc | bb | bb | dd | bb | aa | bb | nn | aa | bb | bb | aa |
| Contorta | cc | bb | bb | bb | dd | bb | ab | bb | aa | aa | bb | bb | aa |
| Cutleaf | bc | bb | bb | bb | dd | bb | aa | bb | cc | ad | bb | ab | bb |
| Pendula | cc | bc | bb | bb | dd | bb | aa | bb | aa | ab | ab | bb | ab |
| Redleaf #3 | cc | bb | bb | bb | cd | bb | ab | ab | nn | aa | bb | bb | aa |
| Rode Zeller | bc | bb | bc | bb | bd | bb | aa | bb | aa | ab | bb | bb | ab |
| Fusco Rubra | cc | bb | bc | ab | dd | bb | aa | bb | cc | aa | bb | bb | aa |
| Purple Aveline | cc | bb | bb | bb | cd | bb | ab | ab | ac | bc | bb | bb | aa |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued).

| Country / Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Aco*1 | *Aco*2 | *Aap*1 | *Aap*2 | *Aap*3 | *Gpi*2 | *Gpi*3 | *Pgm*1 | *Pgm*2 | *Sdh*2 | *Aat* | *Mdh*1 | *Pgd*2 |
| Unnamed Selections | | | | | | | | | | | | | |
| GH 184-19 | bc | bb | bb | bb | ad | aa | aa | aa | aa | aa | bb | bb | ab |
| Hend 5-5 | bb | bb | bb | bb | bd | bb | aa | aa | aa | aa | bb | ab | aa |
| USOR 14-82 | cc | bb | bb | ab | bd | bb | bb | aa | cc | aa | bb | bb | aa |
| USOR 24-82 | cc | bb | bb | ab | ad | bb | bb | nn | cc | aa | ab | bb | ab |
| USOR 26-82 | cc | bb | ac | bb | bc | bb | aa | nn | ac | aa | bb | bb | ab |
| VR 8-32 | bc | bb | bc | bb | dd | bb | bb | ab | aa | aa | ab | bb | aa |
| 17-75 | bc | bb | bb | bb | cd | bb | aa | aa | cc | aa | ab | bb | aa |
| 23-17 | bc | bb | bb | bb | bd | bb | ab | nn | ac | ab | bb | bb | aa |
| 30-13 | cc | bb | bc | bb | bd | bb | ab | bb | nn | aa | bb | bb | bb |
| 39-44 | bc | bb | bb | bb | bd | bb | ab | bb | aa | aa | bb | bb | aa |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued).

| Country / Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Aco*1 | *Aco*2 | *Aap*1 | *Aap*2 | *Aap*3 | *Gpi*2 | *Gpi*3 | *Pgm*1 | *Pgm*2 | *Sdh*2 | *Aat* | *Mdh*1 | *Pgd*2 |
| 43-91 | cc | bb | bb | bb | bc | bb | ab | bb | aa | aa | bb | bb | aa |
| 54-39 | cc | bb | bb | bb | bc | bb | ab | ab | aa | ab | bb | bb | aa |
| 55-77 | ac | bb | bb | bb | bd | bb | ab | aa | ac | ab | bb | bb | aa |
| 55-129 | ac | bb | bb | bb | bd | bb | aa | ab | ac | bd | bb | bb | aa |
| 162-17 | cc | bb | bb | bb | cd | bb | aa | ab | aa | ab | bb | bb | aa |
| 225-77 | bc | bb | bb | bb | bd | bb | aa | aa | aa | aa | bb | bb | aa |
| 226-118 | bc | bb | bb | bb | bd | bb | aa | ab | cc | aa | bb | bb | aa |
| 233-7 | cc | bb | bc | bb | cd | bb | aa | aa | cc | aa | bb | bb | aa |
| 243-2 | bc | bb | bb | bb | dd | bb | aa | aa | aa | ab | bb | bb | aa |
| 246-128 | bc | bb | bb | bb | bd | bb | aa | ab | ac | aa | bb | bb | aa |

Table 5-1. Isozyme phenotypes for 13 polymorphic isozymes in 56 hazelnut cultivars (continued).

| Country / Cultivar | Isozyme Locus | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Aco*1 | *Aco*2 | *Aap*1 | *Aap*2 | *Aap*3 | *Gpi*2 | *Gpi*3 | *Pgm*1 | *Pgm*2 | *Sdh*2 | *Aat* | *Mdh*1 | *Pgd*2 |
| 255-3 | bb | bb | bb | bb | cd | bb | aa | ab | aa | aa | ab | ab | aa |
| 296-82 | bb | bb | bb | bb | dd | bb | aa | aa | cc | aa | bb | bb | aa |

[a]interlocus (one band) between *Gpi*-2 and *Gpi*-3 was present in genotypes with a allele at *Gpi*-2.

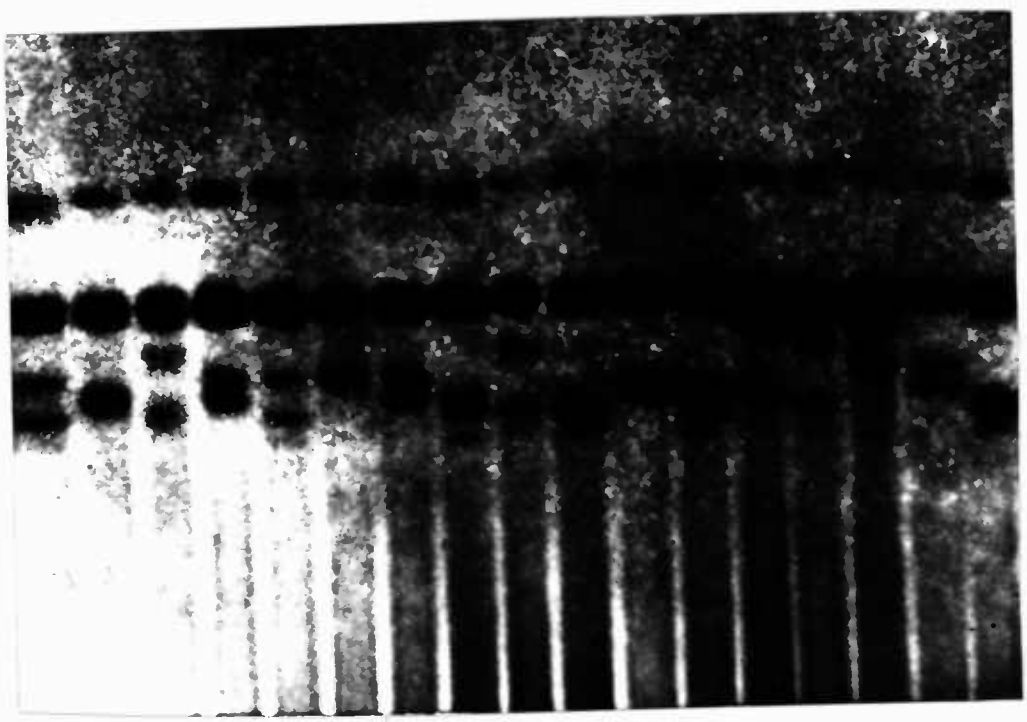

Fig. 5-1. Photogram of AAP isozymes in hazelnut cultivars.

Fig. 5-2. Photogram of AAT isozymes in hazelnut cultivars.

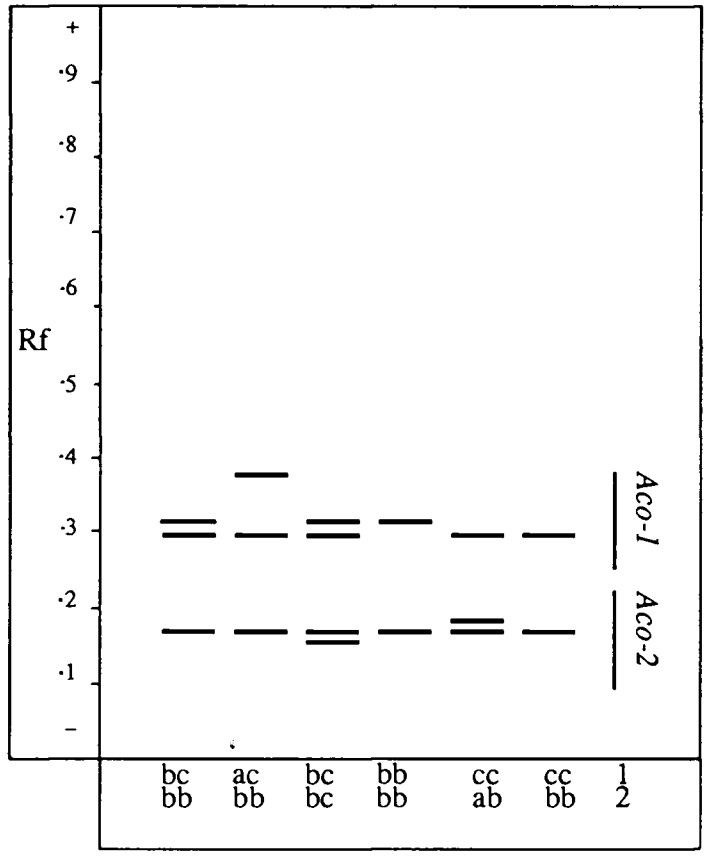


Fig. 5-3. Diagram of ACON isozymes in hazelnut cultivars.

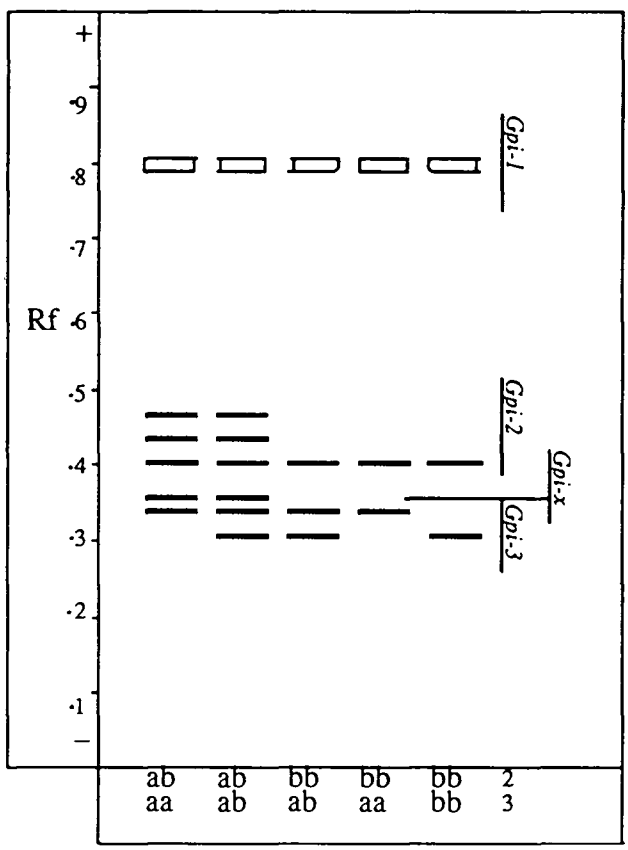


Fig 5-4. Diagram of GPI isozymes in hazelnut cultivars.

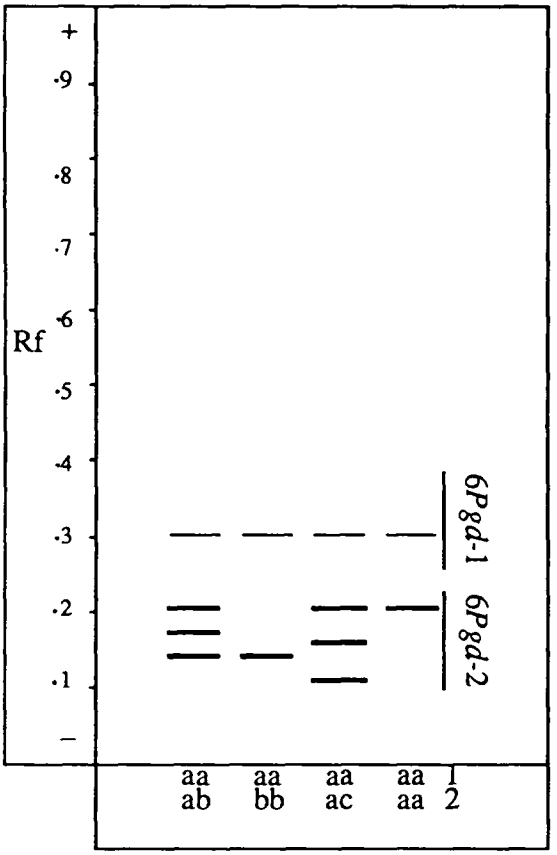


Fig. 5-5. Diagram of 6-PGD isozymes in hazelnut cultivars.

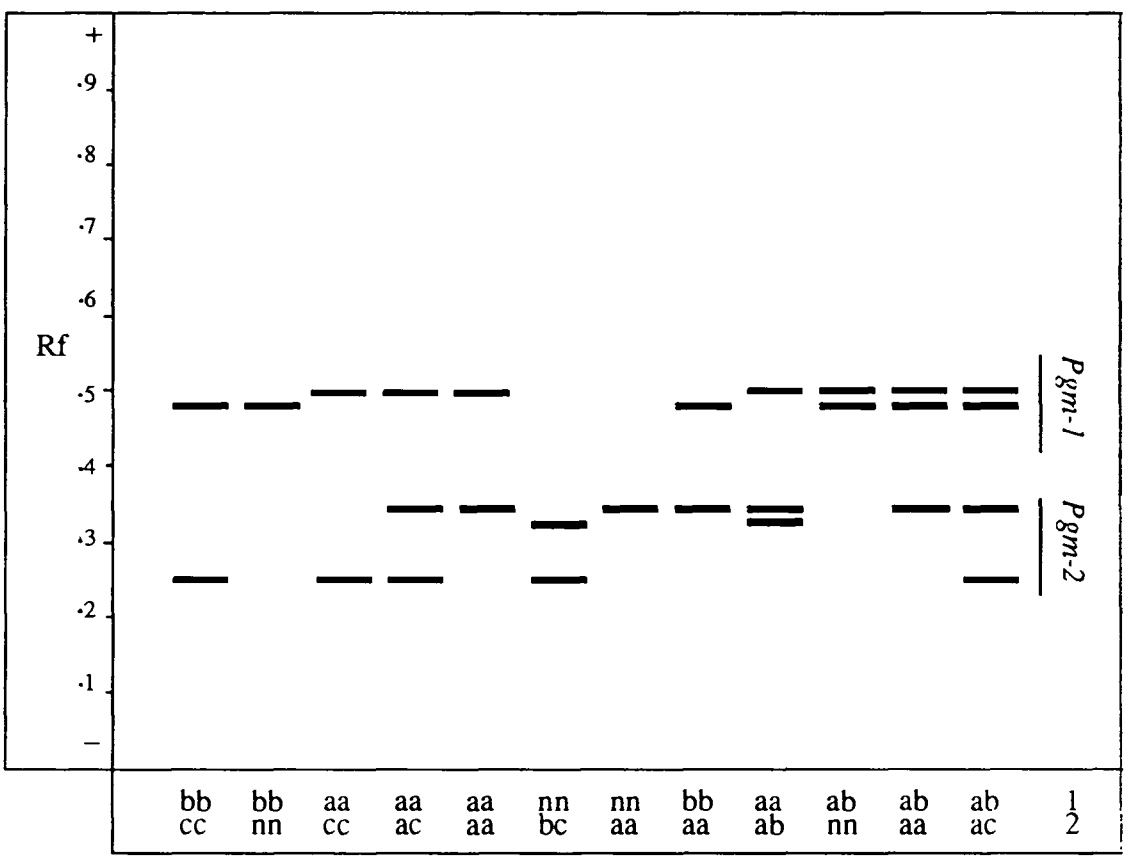


Fig. 5-6. Diagram of PGM isozymes in hazelnut cultivars

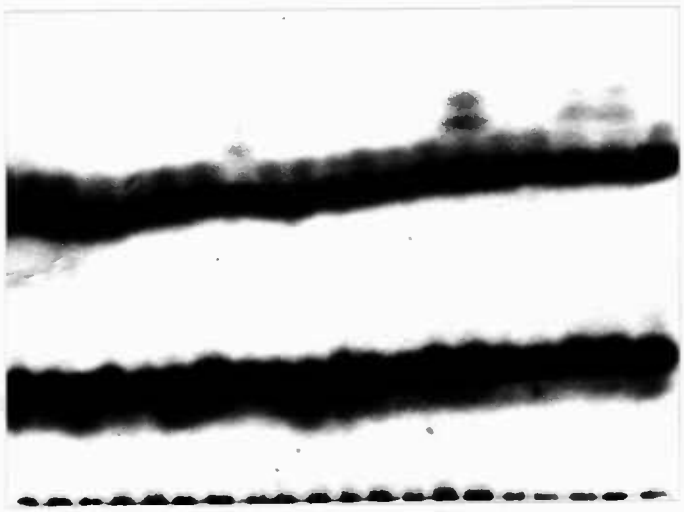

Fig 5-7. Photogram of MDH isozymes in hazelnut cultivars.

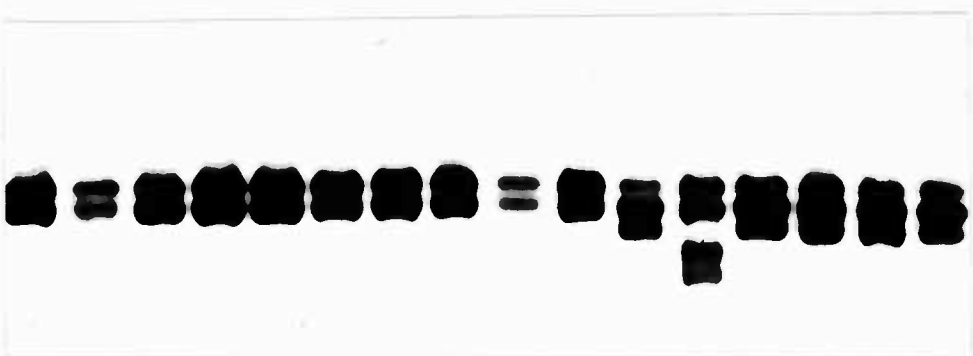

Fig 5-8. Photogram of SDH isozymes in hazelnut cultivars.

## Literature Cited

Ahmad, Z. L., L. S. Daley, R. A. Menendez, and H. B. Lagerstedt. 1987. Characterization of filbert (*Corylus*) species and cultivars using gradient polyacrylamide gel electrophoresis. J. Environ. Hort. 5:11-16.

Arulsekar, S., D. E. Parfitt, and D. E. Kester. 1986. Comparison of isozyme variability in peach and almond cultivars. J. Hered. 77:272-274.

Arulsekar, S., D. E. Parfitt, W. Beres, and P. E. Hansche. 1986. Genetics of malate dehydrogenase isozymes in the peach. J. Hered. 77:49-51.

Byrne, D. H. and T. G. Littleton. 1988. Electrophoretic characterization of diploid plums of the Southeastern United States. J. Amer. Soc. Hort. Sci. 113:918-924.

Byrne, D. H. and T. G. Littleton. 1989. Characterization of isozyme variability in apricots. J. Amer. Soc. Hort. Sci. 114:674-678.

Cerezo, M., R. Socias i Company, and P. Arus. 1989. Identification of almond cultivars by pollen isozymes. J. Amer. Soc. Hort. Sci. 114: 164-169.

Chaparro, J. X., R. G. Goldy, B. D. Mowrey, and D. J. Werner. 1989. Identification of *Vitis vinifera* L. x *Muscadinia rotundifolia* hybrids by starch gel electrophoresis. HortScience 24:128-130.

Cheng, S. Z. and W. H. Yang. 1987. Taxonomic studies of ten species of the genus *Juglans* based on isozymic zymograms. Acta Hort. Sinica

14:90-96.

Degani, C, R. El-Batsri, and S. Gazit. 1990. Enzyme polymorphism in mango. J. Amer. Soc. Hort. Sci. 115: 844-847.

Dewald, M. G., G. A. Moore, and W. B. Sherman. 1988. Identification of pineapple cultivars by isozyme genotypes. J. Amer. Soc. Hort. Sci. 113:935-938.

Durham, R. E., G. A. Moore, and W. B. Sherman. 1987. Isozyme banding patterns and their usefulness as genetic markers in peach. J. Amer. Soc. Hort. Sci. 112:1013-1018.

Hauagge, R., D. E. Kester, S. Arulsekar, D. E. Parfitt, and L. Liu. 1987. Isozyme variation among California almond cultivars: 2. Cultivar characterizations and origins. J. Amer. Soc. Hort. Sci. 112:693-698.

Mehlenbacher, S. A. 1991. Hazelnuts, p. 789-836. In: J. N. Moore and J. R. Ballington (eds.). Genetic resources of temperature fruits and nut crops. Acta Hort. 290.

Menendez, R. A., R. Fritts, and F. E. Larsen. 1984. Identification of apple (*Malus domestica* Borkh.) cultivars by isozyme analysis. HortScience 19:541-543.

Messeguer, R., P. Arus, and M. Carrera. 1987. Identification of peach cultivars with pollen isozymes. Sci. Hort. 31:107-117.

Messina R., R. Testolin, and M. Morgante. 1991. Isozymes for cultivar identification in kiwifruit. HortScience 26:899-902.

Parfitt, D. E., S. Arulsekar, and D. W. Ramming. 1985. Identification of plum x peach hybrids by isozyme analysis. HortScience 20: 246-248.

Tao, R., and A. Sugiura. 1987. Cultivar identification of Japanese persimmon by leaf isozymes. HortScience 22:932-953.

Tsumura, Y., K. Uchida, and K. Ohba. 1989. Genetic control of isozyme variation in needle tissues of *C. japonica*. J. Hered. 80:291-297.

Weeden, N. F. 1989. Applications of isozymes in plant breeding. Plant Breeding Reviews 6:11-39.

Weeden, N. F., and R. C. Lamb. 1985. Identification of apple cultivars by isozyme phenotypes. J. Amer. Soc. Hort. Sci. 110:509-515.

Wolfe, W. H. 1976. Identification of grape varieties by isozyme banding patterns. Amer. J. Enol. Viticult. 27:68-73.

# BIBLIOGRAPHY

Ahmad, Z. L., L. S. Daley, R. A. Menendez, and H. B. Lagerstedt. 1987. Characterization of filbert (*Corylus*) species and cultivars using gradient polyacrylamide gel electrophoresis. J. Environ. Hort. 5:11-16.

Anagnostakis, S. L. 1991. Peroxidase allozyme phenotypes in *Castanea* and their segregation among progeny. HortScience 26:1424.

Anderson C. M., W. S. Castle, and G. A. Moore. 1991. Isozymic identification of zygotic seedlings in swingle citrumelo *Citrus paradisi* x *Poncitrus trifoliata* nursery and field populations. J. Amer. Soc. Hort. Sci. 116:322-326.

Anderson, R. A. and J. A. Sowers. 1968. Optimum conditions for banding of plant phenols to insoluble polyvinylpyrrolidone. Phytochem. 7:293-301.

Arulsekar, S., D. E. Parfitt, and G. H. McGranahan. 1985. Isozyme gene markers in *Juglans* species. Inheritance of GPI and AAT in *J. regia* and *J. hindsii*. J. Hered. 76:103-106.

Arulsekar, S. and D. E. Parfitt. 1986. Isozyme analysis procedures for stone fruit, almond, grape, walnut, pistachio, and fig. HortScience 21:928-933.

Arulsekar, S., D. E. Parfitt, W. Beres, and P. E. Hansche. 1986. Genetics of malate dehydrogenase isozymes in the peach. J. Hered. 77:49-51.

Arulsekar, S., D. E. Parfitt, and D. E. Kester. 1986. Comparison of isozyme variability in peach and almond cultivars. J. Hered. 77:272-274.

Arulsekar, S., G. H. McGranahan, and D. E. Parfitt. 1986. Inheritance of phosphoglucomutase and esterase isozymes in Persian walnut. J. Hered. 77:220-221.

Ayala, F. J. 1975. Genetic differentiation during the speciation process. In: T. Dobzhansky, M. K. Hecht, and W. C. Steere (eds.), Evolutionary Biology, Plenum Press, New York, pp.1-78.

Bendall, D. S. and R. P. F. Gregory. 1963. Purification of phenol oxidase. In: J. B. Pridham (ed.), Enzyme chemistry of phenolic compounds, Pergammon Press, New York.

Bringhurst, R. S., S. Arulsekar, J. F. Hancock Jr., and V. Voth. 1981. Electrophoretic characterization of strawberry cultivars. J. Amer. Soc. Hort. Sci. 106:684-687.

Byrne, D. H. and T. G. Littleton. 1988. Electrophoretic characterization of diploid plums of the Southeastern United States. J. Amer. Soc. Hort. Sci. 113:918-924.

Byrne, D. H. and T. G. Littleton. 1989. Characterization of isozyme variability in apricots. J. Amer. Soc. Hort. Sci. 114:674-678.

Cardy, B. J., C. W. Stuber, and M. M. Goodman. 1980. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Inst. Stat. Mim. Ser. 1317, North Carolina State Univ., Raleigh.

Cerezo, M., R. Socias i Company, and P. Arus. 1989. Identification of almond cultivars by pollen isozymes. J. Amer. Soc. Hort. Sci. 114: 164-169.

Chaparro, J. X., R. G. Goldy, B. D. Mowrey, and D. J. Werner. 1989. Identification of *Vitis vinifera* L. x *Muscadinia rotundifolia* hybrids by starch gel electrophoresis. HortScience 24:128-130.

Cheng, J. S. and D. X. Jia. 1981. Comparison of peroxidase and esterase isozymes among apple cultivars. China Fruit 1:41-44.

Cheng, S. Z. and W. H. Yang. 1987. Taxonomic studies of ten species of genus *Juglans* based on isozymic zymograms. Acta Hort. Sinica 14:90-96.

Cheng S. Z. and W. H. Yang. 1988. Isozymes in fruit trees. J. Hebei Agric. U. 11:50-56

Cheng J. S., J. Z. Niu, and S. Y. Di. 1984. Peroxidase isozymic analysis of apple hybrids. Acta Hort. Sinica 11:73-78.

Chevreau, E., and F. Laurens. 1987. The pattern of inheritance in apple: further results from leaf isozyme analysis. Theor. Appl. Genet. 75: 90-95.

Chevreau, E., Y. Lespinasse, and Gallet. 1985. Inheritance of pollen enzymes and polyploid origin of apple. Theor. Appl. Genet. 71:268-277.

Chyi, Y. S. and N. F. Weeden. 1984. Relative isozyme band intensities permit the identification of the 2N gamete parent for triploid apple

cultivars. HortScience 19:818-819.

Cooper, T. G. 1977. The tools of biochemistry, Wiley-Interscience, New York.

Degani, C., R. El-Batsri, and S. Gazit. 1990. Enzyme polymorphism in mango. J. Amer. Soc. Hort. Sci. 115: 844-847.

Devine T. E., J. J. O'Neill, Y. T. Kiang, and C. J. Bult. 1991. Genetic linkage of the Rj2 gene in soybean. Crop Sci. 31:665-668.

Dewald, M. G., G. A. Moore, and W. B. Sherman. 1988. Identification of pineapple cultivars by isozyme genotypes. J. Amer. Soc. Hort. Sci. 113:935-938.

Dirr, M. A., A. V. Barker, and D. N. Maynard. 1973. Extraction of nitrate reductase from leaves of Esicaceae. Phytochem. 21:1261-1264.

Dirr, M. A., A. V. Barker, and D. N. Maynard. 1972. Nitrate reductase activity in the leaves of the highbush blueberry and other plants. J. Amer. Soc. Hort. Sci. 97:329-331.

Doong, J.-Y. H., and Y.-T Kiang. 1987. Inheritance of aconitase isozymes in soybean. Genome 29:713-717.

Durham, R. E., G. A. Moore, and W. B. Sherman. 1987. Isozyme banding patterns and their usefulness as genetic markers in peach. J. Amer. Soc. Hort. Sci. 112:1013-1018.

Gertz, A. and G. Wricke. 1989. Linkage between the incompatibility locus Z and 1-glucosidase locus in rye. Plant Breeding 102:255-259.

Goldring, A., D. Zamir, and C. Degani. 1985. Duplicated phosphoglucose isomerase genes in avocado. Theor. Appl. Genet. 71:491-494.

Goodman, M. M., C. W. Stuber, K. J. Newton, and H. H. Wessinger. 1980. Linkage relationships of 19 enzyme loci in maize. Genetics 96:697-710.

Gottlieb, L. D. 1882. Electrophoretic evidence and plant populations. Progress in Phytochem. 7:1-46.

Griffin, J. D. and R. G. Palmer. 1987. Inheritance and linkage studies with five

isozyme loci in soybean. Crop Sci. 27:885-892.

Hauagge, R., D. E. Kester, and R. A. Asay. 1987a. Isozyme variation among California almond cultivars : 1. Inheritance. J. Amer. Soc. Hort. Sci. 112:687-693.

Hauagge, R., D. E. Kester, S. Arulsekar, D. E. Parfitt, and L. Liu. 1987b. Isozyme variation among California almond cultivars: 2. Cultivar characterizations and origins. J. Amer. Soc. Hort. Sci. 112:693-698.

Hedges, B. R., J. M. Sellner, T. E. Devine, and R. G. Palmer. 1990. Assigning isocitrate dehydrogenase to linkage group 11 in soybean. Crop Sci. 30:940-942.

Hedrick, P. W. 1983. Genetics of populations. Science Books International, Portola Valley, CA.

Hunt, J. S. and M. F. Barnes. 1982. Molecular diversity and plant disease resistance: an electrophoretic comparison of near-isogenic lines of wilt-resistant or -susceptible *Pisum sativum* L. cv. William Massey. Euphytica 31:341-348.

Hyun, J. O., O. J. Rajora, and L. Zsuffa. 1987. Inheritance and linkage of isozymes in *Populus tremuloides* (Michx.). Genome 29:384-388.

Jarret, R. L. and R. E. Litz. 1986. Enzyme polymorphism in *Musa acuminata* Colla. J. Hered. 77:183-188.

Korban, S. S. and B. L. Bournival. 1987. Catalase, esterase, and peroxidase enzymes in seeds and leaves of *Malus* x *domestica* Borkh. Scientia Hort. 32:213-219.

Labrothe, Ph., S. Poirier-Hamon, and J. Pernes. 1983. Inheritance of leaf peroxidase isozymes in *Nicotiana alata* and linkage with the S-incompatibility locus. Theor. Appl. Genet. 65:163-170.

Landry, B. S., R. V. Kesseli, B. Farrara and R. W. Michelmore. 1987. A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331-337.

Lewontin, R. C. 1974. The genetic basis of evolutionary change. Columbia Univ. Press, New York, 346pp.

Li, C. Z., H. Y. Hua, and M. Z. Xu. 1986. A study on the application of isozyme analysis to the identification of sex in *Actinidia chinensis*. Plant Physiol. Commun. 2:12-13.

Lin, B. N. and D. X. Shen. 1983. Studies of *Pyrus* species characteristics and relationship by isozyme analysis. J. of Zhejiang Agric. U. 3:235-242.

Liu, B. H. and S. J. Knapp. 1990. G-MENDEL: a program for Mendelian segregation and linkage analysis of individual or multiple progeny populations using log-likelihood ratios. J. Hered. 81:407.

Loomis, W. D. and J. Battaile. 1966. Plant phenolic compounds and the isolation of plant enzymes. Phytochem. 5:423-438.

Loukas, M., M. N. Stavrakasis, and C. B. Krimbas. 1983. Inheritance of polymorphic isozymes in grape cultivars. J. Hered. 74:181-183.

Manganaris, A. G., and F. H. Alston. 1987. Inheritance and linkage relations of GOT isozymes in apple: 1. the gene GOT-1, a marker for the S incompatibility locus. Theor. Appl. Genet. 74:154-161.

Manganaris, A. G., and F. H. Alston. 1988a. Inheritance and linkage relations of GOT isozymes in apple: 2. The genes GOT-2 and GOT-4. Theor. Appl. Genet. 76:449-454.

Manganaris, A. G. and F. H. Alston. 1988b. The acid phosphatase gene *Acp-1* and its linkage with the endopeptidase gene *Enp-1* and the pale green lethal gene 1 in apple. Acta Hort. 224:177-184.

Manganaris A. G. and F. H. Alston. 1992a. Genetics of leucine aminopeptidase in apple. Theor. Appl. Genet. 83:345-352.

Manganaris A. G. and F. H. Alston. 1992b. Inheritance and linkage relationships of peroxidase isozymes in apple. Theor. Appl. Genet. 83:392-399.

Markert C. L. and F. Moller. 1959. Multiple forms of enzyme: tissue, ontogenetic and species specific patterns. Proc. Natl. Acad. Sci. 45:753-763.

Marquard, R. D. 1991. Inheritance of phosphoglucomutase isozymes in pecan. HortScience 26:1213-1214.

Marquard, R. D. 1987. Isozyme inheritance, polymorphism, and stability of malate dehydrogenase and phosphoglucose isomerase in pecan. J. Amer. Soc. Hort. Sci. 112:717-721.

Mehlenbacher, S. A. and M. M. Thomspon. 1988. Dominance relationships among S-alleles in *Corylus avellana* L. Theor. Appl. Genet. 76:669-672.

Mehlenbacher, S. A. and M. M. Thompson. 1991. Inheritance of a chlorophyll deficiency in hazelnut. HortScience 26:1414-1416.

Mehlenbacher, S. A., M. M. Thompson, and H. Ronald Cameron. 1991. Occurrence and inheritance of resistance to eastern filbert blight in 'Gasaway' hazelnut. HortScience 26:410-411.

Mehlenbacher, S. A. 1991. Hazelnuts, p. 789-836. In: J. N. Moore and J. R. Ballington (eds.). Genetic resources of temperature fruits and nut crops. Acta Hort. 290.

Mehlenbacher, S. A. and D. C. Smith. 1991. Partial self-incompatibility in 'Tombul' and 'Montebello' hazelnuts. Euphytica 56:231-236.

Menendez R. A. and L. S. Daley. 1986. Characterization of *Pyrus* species and cultivars using gradient polyacrylamide gel electrophoresis. J. Environ. Hort. 4:56-60.

Menendez, R. A., R. Fritts, and F. E. Larsen. 1984. Identification of apple (*Malus domestica* Borkh.) cultivars by isozyme analysis. HortScience 19:541-543.

Messeguer, R., P. Arus, and M. Carrera. 1987. Identification of peach cultivars with pollen isozymes. Sci. Hort. 31:107-117.

Messina R., R. Testolin, and M. Morgante. 1991. Isozymes for cultivar identification in kiwifruit. HortScience 26:899-902.

Mielke, E. A. and W. H. Wolfe. 1982. Identification of pecan cultivars with pollen isozymes. HortScience 17:382-383.

Moore, G. A. and R. E. Litz. 1984. Biochemical markers for *Carica papaya*, *C. cauliflora*, and plants from somatic embryos of their hybrids. J. Amer. Soc. Hort. Sci. 109:213-218.

Mowrey, B. D., D. J. Werner, and D. H. Byrne. 1990. Inheritance of

isocitrate dehydrogenase, malate dehydrogenase, and shikimate dehydrogenase in peach and peach x almond hybrids. J. Amer. Soc. Hort. Sci. 115:312-319.

Muehlbauer, F. J., N. F. Weeden, and D. L. Huffman. 1989. Inheritance and linkage relationships of morphological and isozyme loci in lentil. J. Hered. 80:298-303.

Neilsen, G. 1985. The use of isozymes as probes to identify and label plant varieties and cultivars. In: M. C. Rattazzi, J. G. Scandalios, and G. S. Whitt (eds.), Isozymes: current topics in biological and medical research, Vol. 12, pp. 1-32, Alan R. Liss, Inc., New York.

Parfitt, D. E., S. Arulsekar, and D. W. Ramming. 1985. Identification of plum x peach hybrids by isozyme analysis. HortScience 20: 246-248.

Parfitt, D. E. and S. Arulsekar. 1989. Inheritance and isozyme diversity for GPI and PGM among grape cultivars. J. Amer. Soc. Hort. Sci. 114:486-491.

Peffely, E. B. and C. Orozco-Castillo. 1987. Polymorphism of isozymes within plant introductions of *Allium cepa* L. and *A. fistulosum* L. HortScience 22:956-957.

Pitel, J. A., W. M. Cheliak, and J. Barrett. 1987. Inheritance of allozymes in a black spruce diallele cross. Silvae Genet. 36:149-153.

Pontikis, C. A., M. Loukas, and G. Kousounis. 1980. The use of biochemical markers to distinguish olive cultivars. J. Hort. Sci. 55:333-343.

Quiros, C. F., D. Douches, V. and D'Antonio. 1987. Inheritance of annual habit in celery: cosegregation with isozyme and anthocyanin markers. Theor. Appl. Genet. 74:203-208.

Raymond, D. and L. Weinstraub. 1959. Acrylamide gel as a supporting medium for zone electrophoresis. Science 130:711.

Rennie, B. D. and J. W. Tanner. 1989. Mapping a second fatty acid locus to soybean linkage group 17. Crop Sci. 29:1081-1083.

Rick, C. M. and J. F. Fobes. 1974. Association of an allozyme with nematode resistance. Rep. Tomato Genet. Coop. 24:25.

Roose, M. L. and S. N. Traugh. 1988. Identification and performance of

citrus trees on nucellar and zygotic rootstocks. J. Amer. Soc. Hort. Sci. 113:100-105.

Salesses, G. and A. Bonnet. 1988. Cytogenetic study of hybrids between hazelnut variaties (*Corylus avellana*) carrying a translocation in heterozygous state. (in French). Cytologia 53:407-413

Sanderson, G. M. 1964. Peptidase activity in shoot tips of the tea plant. Biochem. J. 93:419-423.

Santamour, F. S.Jr., A. J. McArdle, and R. A. Jaynes. 1986. Cambial isoperoxidase patterns in *Castanea*. J. Environ. Hort. 4:14-16.

Santamour F. S., Jr. and P. Demuth. 1980. Identification of callery pear cultivars by peroxidase isozyme patterns. J. Hered. 71:447-449.

Schwennesen, J., E. A. Mielke, and W. H. Wolfe. 1982. Identification of seedless table grape cultivars and a bud sport with berry isozymes. HortScience 16:366-368.

Shenoy, V. V., D. V. Seshu, and J. K. Sachan. 1990. Shikimate dehydrogenase-1 allozymes as a marker for high seed protein content in rice. Crop Sci. 30:937-940.

Smed, E., J. P. C. Van Gept, and M. Oleo. 1989. Genetic control and linkage relationships of isozyme markers in sugar beet (*B. vulgaris* L.). Theor. Appl. Genet. 78:97-104.

Soost, R. K., T. E. Williams, and A. M. Torres. 1980. Identification of nucellar and zygotic seedlings of citrus with leaf isozymes. HortScience 15:728-729.

Stavrakakis, M. and M. Loukas. 1983. The between-and within-grape cultivars genetic variation. Scientia Hort. 19:321-334.

Strauss, S. H. and M. T. Conkle. 1986. Segregation, linkage, and diversity of allozymes in knobcone pine. Theor. Appl. Genet. 72:483-493.

Subden, R. E., A. Krizus, S. C. Lougheed, and K. Carey. 1987. Isozyme characterization of *Vitis* species and some cultivars. Am. J. Enol. Vitic. 38:176-181.

Sugiura, A., R. Tao, and T. Tomana. 1988. Distinguishing between Japanese

persimmon cultivars by means of pollen isozymes. Scientia Hort. 36:67-77.

Tanksley, S. D., C. M. Rick, and C. E. Vallejos. 1984. Tight linkage between a nuclear male sterile locus and an enzyme marker in tomato. Theor. Appl. Genet. 68:109-113.

Tanksley, S. D. and T. J. Orton. 1983. Isozymes in plant genetics and breeding, parts A&B, Elsevier, Amsterdam.

Tanksley, S. D. and Loaiza-Figuera F. 1985. Gametophytic self-incompatibility is controlled by a single major locus on chromosome 1 in *Lycopersicon peruvianum*. Proc. Natl. Acad. Sci. 82:5093-5096.

Tao, R., and A. Sugiura. 1987. Cultivar identification of Japanese persimmon by leaf isozymes. HortScience 22:932-953.

Thompson, M. M. 1985. Linkage of the incompatibility locus and red pigmentation genes in hazelnut. J. Hered. 76:119-122.

Thompson, M. M., H. B. Lagerstedt, and S. A. Mehlenbacher. 1992. Hazelnuts. In: J. Janick and J. N. Moore (eds.), Advances in Fruit Breeding, Vol. 2. (in press).

Torres A. M. and B. O. Bergh. 1980. Fruit and leaf isozymes as genetic markers in avocado. J. Amer. Soc. Hort. Sci. 105:614-619.

Torres, A. M., R. K. Soost, and T. Mau-Lastovicka. 1982. Citrus isozymes, genetics and distinguishing nucellar from zygotic seedlings. J. Hered. 73:335-339.

Torres, A. M., T. Mau-Lastovicka, T. E. Williams, and P. K. Soost. 1985. Segregation distortion and linkage of *Citrus* and *Poncirus* isozyme genes. J. Hered. 76:289-294.

Torres, A. M. Fruit Trees. 1983. In: S. D. Tanksley and T. J. Orton (eds.), Isozymes in Plant Genetics and Breeding, Part B, Elsevier, Amsterdam, pp.401-422.

Tsumura, Y., K. Uchida, and K. Ohba. 1989. Genetic control of isozyme variation in needle tissues of *Cryptomeria japonica*. J. Hered. 80:291-297.

Tucker, A. O. and D. E. Fairbrothers. 1970. Extraction of leaf proteins of Mentha for disc electrophoresis. Phytochem. 9:1399-1403.

Valizadeh, M. 1978. Aspects genetiques, ecologiques et agronomiques de l'etude de la variabilite des proteines chez les plantes superieures cas de *Ficus carica* L. Doctoral Thesis, Academie de Montpellier, Univ. des Sci. et Tech. du Languedoc (France), 182pp.

Vallejos, C. E. and S. D. Tanksley. 1983. Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato. Theor. Appl. Genet. 66:241-247.

Vallejos, C. E. and C. D. Chase. 1991. Linkage between isozyme markers and a locus affecting seed size in *Phaseolus vulgaris* L. Theor. Appl. Genet. 81:413-419.

van Heemstra, M.I., L. P. Bruederle, and N. Vorsa. 1991. Inheritance and linkage of thirteen polymorphic isozyme loci in diploid blueberry. J. Amer. Soc. Hort. Sci. 116:89-94.

Vinterhalter, D. V. and D. J. James. 1983. The use of peroxidase polymorphism in the identification of apple scion cultivars. Scientia Hort. 18:253-261.

Vorsa, N., P. S. Manos, and M. I. van Heemstra. 1988. Isozyme variation and inheritance in blueberry. Genome 30:776-781.

Weeden, N. F., R. W. Robinson and F. Ignart. 1984. Linkage between an isozyme locus and one of the genes controlling resistance to watermelon mosaic virus 2 in *Cucurbita ecuadorensis*. Cucuribit Genet. Coop. 7:86-87.

Weeden, N. F., R. Provvidenti, and G. A. Marx. 1984. An isozyme marker for resistance to bean yellow mosaic virus in *Pisum sativum*. J. Hered. 75:411-412.

Weeden, N. F., and R. C. Lamb. 1985. Identification of apple cultivars by isozyme phenotypes. J. Amer. Soc. Hort. Sci. 110:509-515.

Weeden, N. F. 1986. Enzyme loci defined in *Phaseolus vulgaris*. Ann. Rpt. Bean Improv. Coop. 29:53.

Weeden, N. F. and R. C. Lamb. 1987. Genetics and linkage analysis of 19 isozyme loci in apple. J. Amer. Soc. Hort. Sci. 112:865-872.

Weeden, N. F. and R. Provvidenti. 1987. A marker locus, Adh-1, for resistance to pea enation mosaic virus. Pisum Newsl. 19:82-83.

Weeden, N. F., B. I. Reisch, and Mary-Howell E. Maartens. 1988. Genetic analysis of isozyme polymorphism in grape. J. Amer. Soc. Hort. Sci. 113:765-769.

Weeden, N. F. 1989. Applications of isozymes in plant breeding. Plant Breeding Reviews 6:11-39.

Weeden, N. F. and J. F. Wendel. 1989. Genetics of plant isozymes. In: D. E. Soltis and P. S. Soltis (eds.), Isozymes in plant biology, Dioscorides Press, Portland, Oregon, pp.46-72.

Werner D. J. and D. F. Moxley. 1991. Relationship between malate dehydrogenase isozyme genotype and plant vigor in peach. J. Amer. Soc. Hort. Sci. 116:327-329.

Werner, D. J. 1992. Catalase polymorphism and inheritance in peach. HortScience 27:41-43.

Wolfe, W. H. 1976. Identification of grape varieties by isozyme banding patterns. Amer. J. Enol. Viticult. 27:68-73.

Wricke, G. and P. Wehling. 1985. Linkage between an incompatibility locus and a peroxidase isozyme locus in rye. Theor. Appl. Genet. 71:289-291.

Zhu, D. Y. and G. S. Lawes. 1987. Characterization of kiwifruit (*Actinidia deliciosa*) cultivars by gel electrophoresis. Acta Hort. 282:395-398.

# APPENDICES

## Appendix A. Incompatibility Studies

Table A1. Isozyme loci for linkage studies with incompatibility locus.

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| | *Aco*-1 | | | | |
| Tonda Romana x 23-17 | cc x bc | 16bc:10cc | 1:1 | 1.4 | 0.24 |
| Tonda Romana x 23-24 | cc x bc | 17bc:14cc | 1:1 | 0.29 | 0.59 |
| Tonda Romana x TGDL[z] | cc x ac | 13ac :12cc | 1:1 | 0.04 | 0.84 |
| 23-17 x TGDL | bc x ac | 7ab:13ac:25bc:23cc | 1:1:1:1 | 13.70 | 0.003** |
| Willamette x 23-17 | bb x bc | 12bb x 21bc | 1:1 | 2.49 | 0.12 |

Table A1. Isozyme loci for linkage studies with incompatibility locus (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| | *Aco*-2 | | | | |
| Tonda Romana x TGDL | ab x bb | 13ab:12bb | 1:1 | 0.04 | 0.82 |
| Tonda Romana x 23-17 | ab x bb | 10ab:16bb | 1:1 | 1.40 | 0.24 |
| Tonda Romana x 23-24 | ab x bb | 17ab:14bb | 1:1 | 0.29 | 0.59 |
| | *Aap*-3 | | | | |
| 23-17 x TGDL | bd x cd | 9bc:24bd:13cd:27dd | 1:1:1:1 | 12.74 | 0.006** |
| Tonda Romana x 23-24 | cd x bd | 7bc:7bd:11cd:7dd | 1:1:1:1 | 4.34 | 0.23 |
| Tonda Romana x TGDL | cd x cd | 3cc:15cd:8dd | 1:2:1 | 2.98 | 0.23 |

Table A1. Isozyme loci for linkage studies with incompatibility locus (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| | *Mdh*-1 | | | | |
| Willamette x 23-17 | dd x bd | 16bd:10dd | 1:1 | 1.40 | 0.24 |
| Tonda Romana x 23-17 | ab x bb | 15ab:11bb | 1:1 | 0.62 | 0.43 |
| Tonda Romana x 23-24 | ab x bb | 11ab:18bb | 1:1 | 1.71 | 0.19 |
| Tonda Romana x TGDL | ab x bb | 18ab:7bb | 1:1 | 5.00 | 0.03* |
| | *Sdh*-2 | | | | |
| Willamette x 23-17 | aa x ab | 15aa:21ab | 1:1 | 1.01 | 0.32 |
| Tonda Romana x 23-24 | ab x aa | 15aa:16ab | 1:1 | 0.03 | 0.86 |

Table A1. Isozyme loci for linkage studies with incompatibility locus (Contd.).

| Cross | Parental phenotypes | Progeny phenotypes | Expected ratio | G | P |
|---|---|---|---|---|---|
| Tonda Romana x TGDL | ab x ad | 3aa:9ab:8ad:5bd | 1:1:1:1 | 3.87 | 0.26 |
| 23-17 x TGDL | ab x ad | 18aa:12ab:21ad:20bd | 1:1:1:1 | 2.87 | 0.41 |

[z]Tonda Gentile delle Langhe

## Appendix B. Staining Solutions for the Eight enzyme Systems

Table B1. Stain recipes.

| enzyme | Stain buffer | Stain components | |
|---|---|---|---|
| ACON | 75 ml | Cis-aconitic acid | 70 mg |
| Aconitase | 0.2 M tris HCl | NADP | 0.8 ml |
| | pH 8.0 | NBT | 0.8 ml |
| | | PMS | 0.5 ml |
| | | Isocitrate dehydrogenase | 20 units (0.3 ml) |
| AAP | 75 ml | L-alanine 2-naphthylamide | 25 mg |
| Alanine | Aminopeptidase | dissolved in DMSO[x] | 1.5 ml |
| aminopeptidase | buffer | Fast Black K salt | 20 mg |
| | pH 4.5 | | |
| AAT | 75 ml | Aspartate acid | 150 mg |
| Aspartate | 0.2 M phos- | 1-ketoglutarate solution | 2.0 ml |
| aminotransferase | phate buffer | Fast Blue BB salt | 100 mg |
| | pH 7.5 | | |

Table B1. Stain recipes (Contd.).

| enzyme | Stain buffer | Stain components | |
|---|---|---|---|
| 6PGD | 75 ml | 6-phosphogluconic acid | 14 mg |
| 6-phospho- | 0.1 M tris HCl | NADP | 0.8 ml |
| gluconate | | NBT | 0.8 ml |
| dehydrogenase | | PMS | 0.5 ml |
| GPI | 75 ml | D-fructose-6-phosphate | 20 mg |
| Glucose | 0.1 M tris HCl | NADP | 0.8 ml |
| phosphate | pH 8.0 | NBT | 0.8 ml |
| isomerase | | PMS | 0.5 ml |
| | | G6PDH | 20 units |
| PGM | 75 ml | 1-D-glucose-1-phosphate | 50 mg |
| Phospho- | 0.1 M tris HCl | NADP | 0.8 ml |
| glucomutase | pH 8.0 | NBT | 0.8 ml |
| | | PMS | 0.5 ml |
| | | G6PDH | 20 units |

Table B1. Stain recipes (Contd.).

| enzyme | Stain buffer | Stain components | |
|---|---|---|---|
| MDH | 75 ml | Malic solution | 5 ml |
| Malic | 0.1 M tris HCl | NAD | 0.8 ml |
| dehydrogenase | pH 8.0 | NBT | 0.8 ml |
| | | PMS | 0.5 ml |
| SDH | 75 ml | Shikimic acid | 30 mg |
| Shikimate | 0.1 M tris HCl | NADP | 0.8 ml |
| dehydrogenase | pH 8.0 | NBT | 0.8 ml |
| | | PMS | 0.5 ml |

[x]Dimethylsulfoxide

Table B2. Stock solutions for stain components.

| Abbreviation | Name | Concentration |
|---|---|---|
| G6PDH | Glucose-6-phosphate dehydrogenase | 5 units/ml buffer |
| NAD | 2-Nicotinamide adenine dinucleotide | 10 mg/ml water |
| NADP | 2-NAD phosphate | 10 mg/ml water |
| NBT | Nitro blue tetrazolium | 10 mg/ml water |
| PMS | Phenazine methosulfate | 5 mg/ml water |

Table B3. Stain buffer formulation.

| Buffer | pH | Formulation | |
|---|---|---|---|
| Aminopeptidase buffer | 4.5 | Trizma base | 12.1 g |
| | | Maleic anhydride | 9.8 g |
| | | Sodium hydroxide | 1.6 g |
| | | Distilled water | 1.0 liter |
| 0.2 M phosphate buffer | 7.5 | Sodium phosphate, monobasic | 3.84 g |
| | | Sodium phosphate, dibasic | 23.86 g |
| | | Distilled water | 1.0 liter |
| 1.0 M tris hydrochloride | 8.0 | Trizma base | 74.0 g |
| | | Trizma hydrochloride | 61.4 g |
| | | Distilled water | 1.0 liter |