Short Note: Variability and Inheritance of Diaphorases in American and Chinese *Castanea* Species

By F. DANE[1]) and H. HUANG[2])

(Received 21st September 1999)

Summary

The inheritance of DIA isozymes in American and Chinese *Castanea* species was analyzed using agarose isoelectric focusing and the single-tree-progeny method. Two alleles at three DIA loci were found to be codominantly inherited. Genetic variation at *Dia1* was detected in *C. pumila*, and *C. seguinii*, at *Dia2* in *C. dentata*, while genetic polymorphism at *Dia6* was detected in *C. pumila* only. Additional DIA alleles were detected in samples of natural populations. Diaphorase expression at cathodic loci was tissue specific and detectable in cotyledonary tissues mainly.

Key words: Isozyme, *Castanea dentata, C. mollissima, C. pumila, C. seguinii,* chestnut, chinkapin.

Introduction

The genus *Castanea* contains several species, including the American chestnut (*Castanea dentata* [MARSH.] BROKH) and the chinkapin (*C. pumila* [L.] MILLER), which are susceptible to chestnut blight. The pathogen (*Cryphonectria parasitica* [MURRILL] BARR.) was introduced into North America at the beginning of this century and has destroyed mature American chestnut populations and severely affected the closely related chinkapin. Taxonomic studies indicated that chinkapin is one species comprised of two botanical varieties, *C. pumila* var. *pumila* and var. *ozarkensis* (ASHE) TUCKER (JOHNSON, 1988). *C. pumila* var. *pumila* and *C. dentata* are sympatric over a large portion of their ranges, while *C. pumila* var. *ozarkensis* is limited to the Ozark mountains (JOHNSON, 1988).

Considerable efforts have been made to transfer blight resistance from Chinese chestnut (*C. mollissima* BL.) into American chestnut in order to combine resistance with the desirable growth and form qualities of the American chestnut (BURNHAM, 1988; HEBARD, 1994). Any effort to restore *Castanea* species to eastern North American forests would have an increased chance of success if the genetic diversity of each species is known. Allozymes have been used extensively in studies of the genetic diversity, geographical variation and germplasm conservation in natural forest tree populations, because of their codominant nature and low sampling costs (HAMRICK et al., 1992).

The inheritance of several polymorphic isozyme loci has been examined in American and Chinese chestnut species using controlled crosses and the single-tree-progeny method (GILLET, 1991; HUANG et al., 1994a and b). The single-tree-progeny method, a procedure used for species where controlled crosses are not available, proved successful for isozyme genetic studies of chestnut species (HUANG et al., 1994a). However, an understanding of the variability and inheritance of diaphorase (DIA) isozymes in these *Castanea* species has been lacking. The name "diaphorases" or dihydrolipoamide: NAD oxidoreductase (EC 1.8.1.4) has been applied to several enzymes which catalyze the oxidation of either β-NADH or β-NADPH in the presence of an electron acceptor such as 2,6-dichlorophenol-indolphenol (WHITE and WHITE, 1997). DIA has been reported as a tetrameric enzyme in *Castanea sativa* MILL. (FINESCHI et al., 1990), and *Quercus petraea* (MATT.) LIEBL. (ZANETTO et al., 1996), although dimeric and monomeric forms have been described in several other species (WENDEL and WEEDEN, 1989; MURPHY et al., 1990). Our objective was to study the inheritance of DIA variability in American and Chinese *Castanea* species to establish additional isozyme markers for our continuing efforts to evaluate the genetic diversity and population structure in the genus *Castanea* (DANE and HUANG, 1997; HUANG et al., 1998).

Materials and Methods

Single-tree-progeny families derived from *C. pumila* var. *pumila*, and *C. seguinii* DODE, maintained at the Piedmont Substation of the Alabama Agricultural Experiment Station System, and *C. pumila* var. *ozarkensis* from Arkansas were used for genetic analysis (*Table 1*). Open-pollinated seeds were randomly harvested from each parent tree. Single-tree-progeny families derived from *C. dentata*, maintained as trees at the Wagner Research Farm of the American Chestnut Foundation in Meadowview, VA, were kindly provided by F. HEBARD as dormant buds. *C. dentata*, *C. henryi*, populations, and *C. mollissima* cultivars were analyzed using open-pollinated seeds. Enzymes were extracted from diploid (2n=2x=24) cotyledonary tissue or dormant buds using a 2% glycine extraction buffer (pH 8.6) and assayed for DIA using precast agarose isoelectric focusing gels (pH 3 to pH 7) and the staining protocol of WENDEL and WEEDEN (1989). Chi-square tests were used to determine the goodness-of-fit of the segregation ratios to the expected relationships of progeny genotypes (GILLET, 1991).

Results and Discussion

Nuts from several *Castanea* species showed polymorphic DIA zones, some with tissue-specific expression. Single or five-banded patterns were found at at least three loci. Segregation at the most anodic locus, *Dia1*, was detected only in two species, *C. pumila* vars. *pumila*, and *ozarkensis* and *C. seguinii*, confirming the tetrameric nature of the isozyme and concurring with results observed in the European chestnut species, *C. sativa* MILL. (FINESCHI et al., 1990). Chi-square test results listed in *table 2* are consistent with the hypothesis that one locus with at least two codominant alleles control the variants of the isozyme phenotypes (*Figure 1*). Results from one single-tree progeny test (*Table 2:* 8 to 18) caused rejection of the expected relationship between observed and expected offspring phenotypes, which can be attributed to chance alone. Additional alleles were detected in single tree progenies of both species at low frequencies. No variation at the *Dia1* locus was detected in the other *Castanea* species. *C. dentata* showed the

[1]) Department of Horticulture, Auburn University, Auburn, AL 36849, U.S.A.

[2]) Wuhan Institute of Botany, The Chinese Academy of Science, Wuhan, Hubei 430074, P.R. China

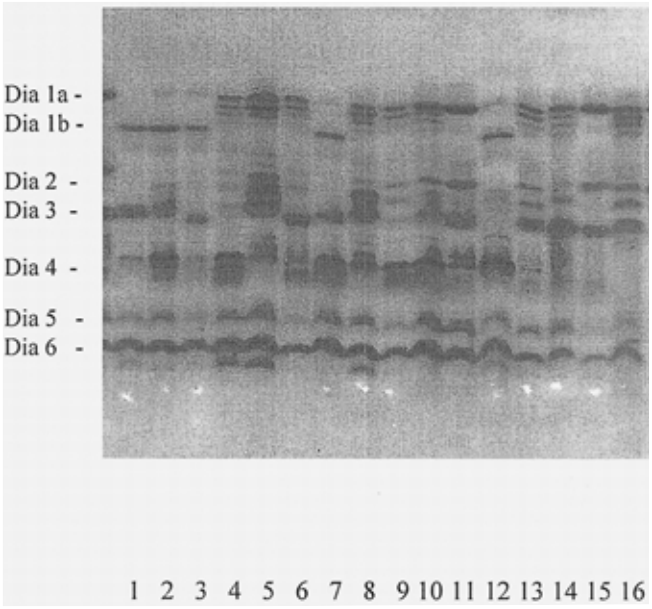

Figure 1. – Diaphorase isozyme banding patterns in *C. pumila* var. *pumila* (single tree-progeny test) with the *Dia1* aa genotype evident in lane 11 & 15, *Dia1* bb in lanes 1–3, 7, 12, and the heterozygous genotype in lanes 4–6, 8–10, 13, 14, and 16.

aa phenotype, while the bb phenotype was found in *C. mollissima* cultivars (*Table 1*) and *C. henryi*. Variation at the other loci (DIA2-DIA5) was observed in *C. dentata*, *C. pumila* var. *pumila* and *ozarkensis*, *C. seguinii* and *C. mollissima*. Clear segregation patterns could only be detected at the *Dia2* locus in *C. dentata*, which can be explained by the presence of 2 codominant alleles expressing tetrameric variation (*Table 2*). Lack of clear-cut segregation patterns at DIA2 to DIA5 in the other species could be attributed to developmental and environmental effects.

Tetrameric variation at the *Dia6* locus was detected only in nuts of *C. pumila* var. *ozarkensis*. The quantitative single-tree-progeny test (GILLET, 1991) could be used for one population sample only, where many nuts had been collected from a small population of Ozark chinkapin trees in Newton County, AR, and was indicative of two codominant alleles at one *Dia6* locus (*Table 2*). All other *Castanea* species expressed the aa phenotype in cotyledon tissues. Expression of cathodic diaphorases was tissue specific, mostly absent from vegetative tissues (DANE, unpublished results), but clearly expressed in cotyledonary tissues.

Our focus has been to assess the geographic variation and genetic diversity differences of wild American *Castanea* populations in order to effectively determine conservation strategies for these species. Knowledge about the presence and inheritance of additional allozymes should afford an increased understanding of the diversity of *Castanea* species and provide additional molecular markers for the assessment of gene flow between these populations.

Literature Cited

BURNHAM, C. R.: The restoration of the American chestnut. Am. Scientist **76**: 478–487 (1988). — DANE, F. and HUANG, H.: Evaluation of the genetic diversity *Castanea pumila* var. *ozarkensis* through isozyme analysis and DNA amplification fingerprinting. Hortscience **32**: 441 (1997). — FINESCHI, S., GILLET, E. and MALVOLTI, M. E.: Genetics of sweet chestnut (*Castanea sativa* MILL). 3. Genetic analysis of zymograms of single tree offspring. Silvae Genetica **39**: 5–6 (1990). — GILLET, E.: Genetic analysis using single tree progenies. In: S. FINESCHI, M. E. MALVOLTI, F. CANNATA and H. H. HATTEMER (eds.). Biochemical markers in the population genetics of forest trees. SPB Academic Publishing bv, The Hague, The Netherlands (1991). — HAMRICK, J. L., GODT, M. J. W. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**: 95–124 (1992). — HEBARD, F. V.: Inheritance of juvenile leaf and stem morphological traits in crosses of Chinese and American chestnut. J. Heredity **85**: 440–446 (1994). — HUANG, H., DANE, F. and KUBISIAK, T. L.: Allozyme and RAPD analysis of the genetic diversity and geographic variation in wild populations of the American chestnut *Castanea dentata* (Fagaceae). Amer. J. Bot. **85**: 1013–1021 (1998). — HUANG, H., DANE, F. and NORTON, J. D.: Genetic analysis of 11 polymorphic isozyme loci in chestnut species and characterization of chestnut cultivars by multi-locus allozyme genotypes. J. Amer. Soc. Hort. Sci. **119**: 840–849 (1994a). — HUANG, H., DANE, F. and NORTON, J. D.: Allozyme diversity in Chinese, Seguine and American chestnut *(Castanea* spp.). Theor. Appl. Genet. **88**: 981–985 (1994b). — JOHNSON, G. P.: Revision of *Castanea* sect. Balanocastanon (Fagaceae). J. Arnold Arboretum **69**: 25–49 (1988). — MURPHY, R. M., SITES, J. W.,

Table 1. – Source, origin and sample size of *Castanea* plant material.

Species	Source	Origin	Samples per population
C. dentata	Troy, NY	Troy, N.Y.	8 nuts
*C. dentata**	Wagner Research Farm, Meadowview, VA	Glade Mountain, VA	12 buds per single-tree-progeny
C. mollissima cultivars: 'AU-Cropper', 'Thin-bur', 'Carolina', 'Homestead', 'Black Beauty', 'AU-Leader'	AU Chestnut Orchard, Auburn University, AL	P.R. China	12 nuts per cultivar
*C. seguinii**	Piedmont Substation, Camp Hill, AL	Chinese Chestnut Germplasm Plantation, Hubei Province, P.R. China	20-40 nuts per single-tree-progeny
C. *pumila* var. *pumila**	Piedmont Substation, Camp Hill, AL	Eglin Air Force Reservation, FL	19-35 nuts per single-tree progeny
C. pumila var. *ozarkensis**	Ozark National Forest, AR	Ozark National Forest, AR	20-36 nuts per population
C. henryi	Hubei Province, P.R. China	Hubei Province, P.R. China	24 nuts

* Populations used for genetic analyses

Table 2. – Genetic analysis of DIA isozyme loci using single-tree-progeny in *Castanea* species.

Species	Locus	maternal tree		Progeny genotype						Expected progeny relationship (Gillet, 1991)	χ^2
		name	genotype	total	aa	ab	bb	ac	bc		
C. seguinii	***Dia1***	7-12	ab	40	4	17	17	1	1	$N_{ab}=N_{aa}+N_{bb}$	0.40
C. seguinii	***Dia1***	9-15	bb	20		2	18			$N_{b.}$	
C. seguinii	***Dia1***	7-33	bb	20		1	19			$N_{b.}$	
C. pumila var. pumila	***Dia1***	8-4	ab	20	5	12	3			$N_{ab}=N_{aa}+N_{bb}$	0.80
C. p. var. *pumila*	***Dia1***	8-18	ab	35	12	11	12			$N_{ab}=N_{aa}+N_{bb}$	4.80*
C. p. var. pumila	***Dia1***	8-29	ab	23	5	9	9			$N_{ab}=N_{aa}+N_{bb}$	1.10
C. p. var. pumila	***Dia1***	8-22	ab	24	3	8	12		1	$N_{ab}=N_{aa}+N_{bb}$	2.13
C. p. var. pumila	***Dia1***	8-21	ab	19	2	12	5			$N_{ab}=N_{aa}+N_{bb}$	1.40
C. p. var. pumila	***Dia1***	8-15	ab	16	4	8	3		1	$N_{ab}=N_{aa}+N_{bb}$	0.07
C. dentata	***Dia2***	T1	ab	12	3	5	4			$N_{ab}=N_{aa}+N_{bb}$	0.33
C. dentata	***Dia2***	T2	ab	12	5	3	4			$N_{ab}=N_{aa}+N_{bb}$	3.00
C. dentata	***Dia2***	T3	ab	12	3	5	4			$N_{ab}=N_{aa}+N_{bb}$	0.33
C. pumila var. *ozarkensis*	***Dia6***	Newton Ct, AR	ab	28	3	14	11			$N_{ab}=N_{aa}+N_{bb}$	0

* Significant at P=0.05 level.

Jr., BUTH, D. G. and HAUFLER, C. H.: Protein 1: Isozyme electrophoresis. In: D. M. HILLS and C. MORITZ (eds.). In: Molecular systematics. Sinauer Associates, Inc. Publishers, Sunderland, MA. (1990). — WENDEL, J. F. and WEEDEN, N. F.: Visualization and interpretation of plant isozymes. In: D. E. SOLTIS and P. S. SOLTIS (eds.). Isozymes in plant biology. Dioscorides Press, Portland, OR. (1989). — WHITE, J. S. and WHITE, D. C.: Sourcebook of enzymes. CRC. Press, Inc. p. 9–33 (1997). — ZANETTO, A., KREMER, A., MULLER-STARCK, G. and HATTEMER, H. H.: Inheritance of isozymes in pedunculate oak (*Quercus robur* L.). J. Heredity **87**: 364–370 (1996).

Short Note: Hermaphrodism in Black Pine

By D. MATZIRIS[1])

(Received 31st March 2000)

Summary

During 1990, a ramet of a clone was observed bearing a small proportion (18 %) of hermaphroditic strobili, in a 12 year-old clonal seed orchard of black pine (*Pinus nigra* ARNOLD), located in central west of Peloponnesos Greece. There are indications that this anomaly is genetically controlled. However research is now in progress to delineate if and how the characteristic is inherited.

Key words: Hermaphrodism, black pine, ramet, strobilus, diallel design.

In nearly all the gymnosperms male and female strobili occur on the same tree. The male strobili are borne in clusters at the base of the twig bud, and the female strobili are borne in groups of one and usually more conelets at the apex of the bud. The female conelet consists of an axis upon which ovuliferous scales are arranged. On the upper face of the ovuliferous scales of the conelet are located two ovules with inverted their micropyle faces towards the axis of the conelet. However there have been various anomalies of the patterns of organization of the female strobili known to occur. (ZOBEL and GODDARD, 1954; DORMAN, 1974).

[1]) National Agricultural Research Foundation,
Forest Research Institute,
Terma Alkmanos,
Athens 11528, Greece