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Isozyme inheritance and variation in *Actinidia*

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Isozyme inheritance and variation in *Actinidia* was investigated using 23 enzyme systems. Ten isozyme loci from six enzyme systems, *Acp-2*, *Est*, *Prx-1*, *Prx-2*, *Prx-3*, *Prx-4*, *Prx-5*, *Pgi-2*, *Pgm-2* and *Tpi*, were found to be inherited as single Mendelian genes in families of two interspecific crosses. Disomic inheritance detected at 10 loci in progenies of a cross between the hexaploid *A. deliciosa* and the diploid *A. chinensis* (Jingkui × Moshan no. 4) provided convincing evidence that *A. deliciosa* is an allohexaploid. Allelic segregation for tetrasomic inheritance at the 10 isozyme loci was demonstrated in the progenies of a cross between the tetraploid *A. chinensis* and the diploid *A. eriantha*, a result suggesting autoploid origin of the tetraploid *A. chinensis*, which apparently originated from its diploid ancestor *A. chinensis*. A high level of isozyme variation and heterozygosity was observed in the 22 cultivars and 56 plants from 28 *Actinidia* taxa. Allozyme phenotype can be used effectively for cultivar identification. The application of isozyme markers in the study of phylogeny could still be difficult in polyploids of natural populations in which estimations of gene frequencies are a prerequisite.

Keywords: *Actinidia*, disomic inheritance, isozyme loci, kiwifruit, polyploid, tetrasomic inheritance.

Introduction

The genus *Actinidia* contains more than 60 species and 110 taxa (Cui, 1993). The best-known species is *A. deliciosa* C. F. Liang et A. R. Ferguson, the commercially developed kiwifruit. The natural range of *Actinidia* is remarkably wide, as it extends from the tropics (latitude 0°) to cold temperate regions (50°N). *Actinidia* species are found from Siberia to Indonesia, and from India to Japan (Cui, 1993). However, the original distribution of most taxa of *Actinidia* is centred around the mountains and hills of south-central and south-east China, with the QinLing mountain as the northern boundary and the HengDuan mountain as the western boundary. Fifty-nine species, 43 varieties and seven forms have been found in China (Liang, 1983; Ferguson, 1990) Species identification in *Actinidia* has proven very difficult because morphological differences between species and intraspecific taxa are not always clear-cut (Ferguson, 1990). The overlapping geographical distributions and natural hybridization of taxa have also produced various transitional forms between varieties of the same species.

All species of *Actinidia* are dioecious but show variations in ploidy level. Diploid ($2n = 58$), tetraploid ($2n = 4x = 116$) and hexaploid ($2n = 6x = 174$) species are known. In several species, there is evidence for intrataxon variation in ploidy level (Xiong *et al.*, 1985; Yan *et al.*, 1994). Dioecism and ploidy level variation greatly hinder the genetic study of heritable traits, particularly flower and fruit traits, which are usually very useful for the identification of cultivars and species. Although *A. deliciosa* (6x) is well known throughout the world as a successful commercial crop, inheritance of most of its important traits remains unknown so far. Also, the mode of inheritance of the commercially important species, such as *A. deliciosa* (6x) and *A. chinensis* (2x, 4x), remains unclear. The majority of the breeding programmes for cultivar improvement depend largely on extensive selections from natural germplasm populations. A national germplasm repository of *Actinidia* and breeding programme for kiwifruit cultivar improvement were initiated in 1978 at the Wuhan Institute of Botany with, as goals, the

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preservation of natural resources of *Actinidia* in China and the development of superior cultivars with higher quality, stress tolerance and pest resistance. It is, therefore, important to develop simply inherited markers, such as isozymes, for studying genetics, because a knowledge of chromosome behaviour and mode of inheritance at different ploidy levels is a prerequisite for breeding strategies and will also provide information about phylogenetic relationships within the genus *Actinidia*.

Isozymes offer several applications in studies of both intraspecific and interspecific phylogenetic systematics (Murphy *et al.*, 1990). Isozymes have also been used extensively in other fruit tree breeding programmes as genetic markers for the identification of cultivars (Degani *et al.*, 1995), the confirmation of hybridity (Anderson *et al.*, 1991), marker-assisted selection (Manganaris *et al.*, 1994) and many other aspects of plant breeding programmes (Tanksley & Orton, 1983). However, no attempt has been made to study isozymic inheritance and variations in *Actinidia*. The only published work to date reported the use of aspartate aminotransferase (AAT), phosphoglucomutase (PGM) and phosphoglucoisomerase (PGI) for the identification of nine kiwifruit cultivars (Messina *et al.*, 1991). The purpose of the present study was (i) to establish isozyme markers for *Actinidia* species by progeny testing of controlled crosses and (ii) to evaluate isozyme variation in a wide array of accessions of *Actinidia* in our repository.

Materials and methods

Plant material

Progenies derived from two interspecific crosses were used for genetic analysis. The JM family (106 progenies) was developed from the controlled cross between the pistillate cultivar Jingkui (6x) of *A. deliciosa* and the staminate cultivar Moshan no. 4 (2x) of *A. chinensis* Planch. These two species are closely related and completely cross-compatible. The WE family (60 progenies) was developed by crossing a staminate plant of *A. eriantha* Benth. (2x) with the pistillate cultivar Wuzhi no. 3 of *A. chinensis* (4x). The hybridization techniques for interspecific crosses in *Actinidia* have been described previously by Wang *et al.* (1994). Twenty-eight taxa representing three sections of the genus (Table 4) were included in this study for the evaluation of isozyme variability. *Actinidia deliciosa* and *A. chinensis*, however, were represented by 10 and 12 cultivars respectively (Table 3). Typically, one male and one female plant of each taxon was used for analysis. Plants of all taxa and the progenies were at least 2 years old. The plants were grown at a 3×4 m spacing in experimental field plots at the Wuhan Institute of Botany, Wuhan, China.

Electrophoresis and isozyme staining

Isozyme assays were conducted on winter dormant buds. The extraction procedure, as described by Huang *et al.* (1994), and the isoelectric focusing polyacrylamide slab gel system of pH 4–9, as described by Mulcahy *et al.* (1981), were used in this study because of their high resolution and efficiency (100 samples per run), with the exception of phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM) assays, which were conducted on 12 per cent starch gels with a pH 6.1 morpholine–citrate buffer (Wendel & Weeden, 1989). Gels were assayed for acid phosphatase (ACP; EC 3.1.3.2), alcohol dehydrogenase (ADH; EC 1.1.1.1), aconitate hydratase (ACO; EC 4.2.1.3), adenylate kinase (ADK; EC 2.7.4.3), esterase (EST; EC 3.1.1.-), formate dehydrogenase (FDH; EC 1.2.1.2), fructose-bisphosphate aldolase (FBA; EC 4.1.2.13), fructose bisphosphatase (FBP; EC 3.1.3.11), fumarate hydratase (FUM; EC 4.2.1.2), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), glutamate dehydrogenase (GDH; EC 1.4.1.2), glyceraldehyde-3-phosphate dehydrogenase (G3PDH; EC 1.2.1.12), hexokinase (HEX; EC 2.7.1.1), isocitrate dehydrogenase (IDH; EC 1.1.1.41), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.40), mannose-6-phosphate isomerase (MPI; EC 5.3.1.8), peroxidase (PRX; EC 1.11.1.7), phosphoglucomutase (PGM; EC 5.4.2.2), phosphogluconate dehydrogenase (PGD; EC 1.1.1.44), phosphoglucoisomerase (PGI; EC 5.3.1.9), shikimate dehydrogenase (SKD; EC 1.1.1.25) and triose-phosphate isomerase (TPI; EC 5.3.1.1). Staining recipes were those of Wendel & Weeden (1989), with minor pH and ingredient concentration modifications.

Allozyme designation and data analysis

The genetic control of isozyme loci was postulated based on the phenotypic banding patterns and the segregation ratios of the controlled crosses. The loci for a given multilocus enzyme system were designated sequentially by number starting with *1* for the most anodal locus; at each locus, the alleles were designated sequentially by letter starting with *a* for the most anodal allozyme. Allozymic phenotypes were designated by capital letters corresponding to

the same alleles (such as phenotype AB corresponding to *ab*, *aaab*, *aabb* or *abbb* genotypes). Irregularly spaced rare bands found occasionally in some taxa were designated as rare allele (R) by number starting with 1 for the most anodal allele (such as R1). Chi-square tests were used to determine the goodness-of-fit of segregating loci to expected Mendelian segregation ratios. In case of a two-class segregation ratio, chi-squares were calculated using Yates' correction.

Results and discussion

Inheritance and variation

Of the 23 enzymes assayed in this study, seven (ACP, EST, MDH, PRX, PGI, PGM and TPI) produced high-resolution banding patterns. The following 16 enzymes were either poorly resolved or of weak intensity and were excluded from further discussion: ADH, ACO, ADK, FDH, FBA, FBP, FUM, G3PDH, G6PDH, GDH, HEX, IDH, ME, MPI, PGD and SKD. Polymorphism was observed at all of the seven well-resolved enzyme systems. Genetic inferences made to explain the variability at these enzyme systems were based on the banding patterns observed in the segregating progenies. However, for MDH, segregations were not observed in the crosses, and the complex zymogram patterns of the cultivars and species provided no inference about its genetics. ACP, EST, PGM and PRX were found to be monomeric enzymes, whereas PGI and TPI were dimeric enzymes in *Actinidia*. The inheritance at each locus and the variation detected among

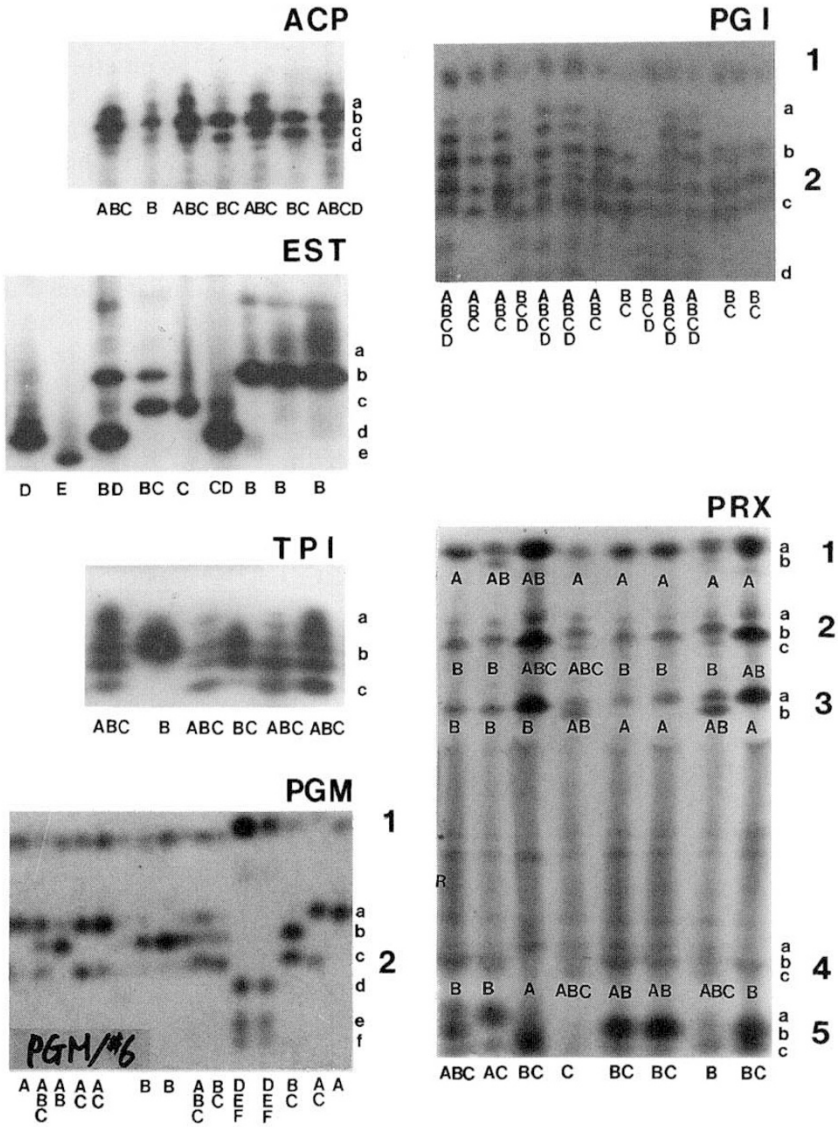

Fig. 1 Allele and locus designations and allozyme phenotypes of 12 isozyme loci in *Actinidia*.

cultivars and different species will be discussed below.

Esterase (EST)

In the JM family [*A. deliciosa* (6x) × *A. chinensis* (2x)], progenies segregated for either single- or double-banded phenotypes and gave the expected Mendelian segregation ratio of 1:2:1 (Fig. 1 and Table 1). This result is consistent with the disomic mode of inheritance of an allohexaploid species. Analysis of one repeat DNA sequence in *A. deliciosa* recently suggested the alloploid nature of this species (Crowhurst & Gardner, 1991). In the WE family [*A. chinensis* (4x) × *A. eriantha* (2x)], three banding patterns, single-, double- and triple-banded, were observed in the progenies. Segregation data for this family fit the expected phenotypic ratios for a tetrasomic mode of inheritance of three alleles at this locus (Table 2). Both diploid and tetraploid plants have been found in *A. chinensis* (Yan *et al.*, 1994). The tetrasomic inheritance of the *Est* allozymes points to the autoploid origin of the tetraploid *A. chinensis* from a diploid *A. chinensis*.

Table 1 Segregation and χ^2 goodness-of-fit test for disomic inheritance at 10 isozyme loci in the JM family from the interspecific cross *Actinidia deliciosa* × *A. chinensis*

	Parental					
Locus	Phenotype	Genotype	Expected gamete ratios	Expected phenotypic ratios	Progeny phenotypes	χ^2
Acp-2	BC × B	----*bc*† × *bb*	(1--*b*:1--*c*) × (*b*)	1B:1BC	58B:43BC:5ABC?‡	2.481 NS
Est	BC × BC	----*bc* × *bc*	(1--*b*:1--*c*) × (1*b*:1*c*)	1B:2BC:1C	22B:50BC:34C	3.057 NS
Pgi-2	ABC × BC	*abc-bc* × *bc*	(1*abc*:1-*bc*) × (1*b*:1*c*)	1ABC:1BC	58ABC:48BC	0.943 NS
Pgm-2	BCD × BD	*-cd-cd* × *bd*	(1-*cb*:1-*cd*) × (1*b*:1*d*)	1BC:2BCD:1CD	17BC:63BCD:26CD	5.303 NS
Prx-1	AB × AB	----*ab* × *ab*	(1--*a*:1--*b*) × (1*a*:1*b*)	1A:2AB:1B	25A:55AB:26B	0.169 NS
Prx-2	A × B	-----*a* × *bb*	(--*a*) × (*b*)	AB	106AB	
Prx-3	AB × A	----*ab* × *aa*	(1--*a*:1--*b*) × (*a*)	1A:1AB	51A:55AB	0.151 NS
Prx-4	BC × C	----*bc* × *cc*	(1--*b*:1--*c*) × (*c*)	1BC:1C	55BC:51C	0.151 NS
Prx-5	B × C	-----*b* × *cc*	(--*b*) × (*c*)	BC	106BC	
Tpi	BC × BC	----*bc* × *bc*	(1--*b*:1--*c*) × (1*b*:1*c*)	1B:2BC:1C	28B:49BC:29CC	0.623 NS

†Indicates a null or monomorphically duplicated allele.
‡Unexpected phenotype class.

Table 2 Segregation and χ^2 goodness-of-fit test for tetrasomic inheritance at 10 isozyme loci in the WE family from the interspecific cross *Actinidia chinensis* × *A. eriantha*

	Parental					
Locus	Phenotype	Genotype	Expected gamete ratios	Expected phenotypic ratios	Progeny phenotypes	χ^2
Acp-2	BC × D	*bcbc* × *dd*	(1*bb*:4*bc*:1*cc*) × (*d*)	1BD:4BCD:1CD	3BD:45BCD:6CD:6BC?†	7.125*
Est	BC × AC	*bccc* × *ac*	(1*bc*:1*cc*) × (1*a*:1*c*)	1AC:1ABC:1BC:1CC	8AC:14ABC:16BC:22C	6.667 NS
Pgi-2	CDE × CE	*ccde* × *ce*	(1*cc*:2*cd*:2*ce*:1*de*) × (1*c*:1*e*)	1C:2CD:5CE:3CDE:1DE	8C:8CD:25CE:17CDE:0DE	7.467 NS
Pgm-2	BD × B	*bbdd* × *bb*	(1*bb*:4*bd*:1*dd*) × (*b*)	1B:5BD	13B:41BD:3BCD?:3BC?	2.070 NS
Prx-1	AB × A	*aaab* × *aa*	(1*aa*:1*ab*) × (*a*)	1A:1AB	37A:27AB	2.817 NS
Prx-2	ABC × BC	*abbc* × *bc*	(2*ab*:2*bc*:1*bb*:1*ac*: × (1*b*:1*c*)	1AC:2AB:5BC:3ABC:1B	5AC:17AB:19BC:17ABC:5B	6.607 NS
Prx-3	A × AB	*aaaa* × *ab*	(*aa*) × (1*a*:1*b*)	1A:1AB	36A:24AB	2.017 NS
Prx-4	ABC × AB	*aabc* × *ab*	(1*aa*:2*ab*:2*ac*:1*bc*) × (1*a*:1*b*)	1A:3ABC:5AB:2AC:1BC	6A:14ABC:26AB:9AC:5BC	0.407 NS
Prx-5	ABC × BC	*abcc* × *bc*	(1*ab*:2*ac*:2*bc*:1*cc*) × (1*b*:1*c*)	1AB:3ABC:5BC:2AC:1C	3AB:14ABC:29BC:11AC:3C	2.407 NS
Tpi	ABC × A	*abcc* × *aa*	(1*ab*:2*ac*:2*bc*:1*cc*) × (*a*)	1AB:3AC:2ABC	11AB:32AC:17ABC	1.883 NS

†Unexpected phenotype class.
*Significant at $P < 0.05$.

Theoretically, autotetraploids can carry up to four different alleles per locus. The triallelic genotype expressed as a triple-banded phenotype simply indicates the monomeric nature of the *Est* locus. The genetic designation of the *a*, *b* and *c* alleles was based on results from the two crosses. Of the 22 cultivars examined, 55 per cent of the cultivars were heterozygous for the *b* and *c* alleles and showed the BC phenotype (*bbcc*, *bbbc*, *bccc*) (Table 3). Allozymic genotyping of these heterozygotes requires additional information from cytogenetic studies and allelic segregation analysis from controlled crosses. Of the 28 taxa in which one male and one female plant were examined, the *a* allele was found in three taxa only: *A. callosa* var. *discolor*, *A. zhejiangensis* and *A. grandiflora*. Two additional cathodal bands, putatively designated alleles *d* and *e* (cathodal to *c*), were observed in five taxa (*A. cylindrica* var. *reticulata*, *A. fulvicoma*, *A. callosa* var. *discolor*, *A. melanandra* and *A. arguta*) and three taxa (*A. polygama*, *A. callosa* var. *discolor and A. valvata*), respectively (Fig. 1 and Table 4). The majority of plants were heterozygotes and had the *b* allele in common, whereas 32 per cent and 9 per cent of plants were found to be homozygous for the *c* and *b* alleles, respectively. This *Est* locus appears to be homologous in all *Actinidia* species.

Acid phosphatase (ACP)

One polymorphic zone, consisting of either single- or double-banded phenotypes, was observed in the progenies of JM (*A. deliciosa* × *A. chinensis*), and an additional triple-banded phenotype was observed in the WE (*A. chinensis* × *A. eriantha*) family. The expected phenotypic ratio of the JM family followed a disomic inheritance pattern (Table 1), whereas segregation data for the WE family, in which the expected phenotypic ratio was tested for tetrasomic inheritance, were skewed with marginal significance at the 5 per cent level (Table 2). This can be attributed to preferential segregation in an interspecific cross between less related species, as 10 per cent of an unexpected maternal phenotype (BC) was found in the progenies. A more anodal band was also detected among the cultivars and species. Four alleles can be tentatively designated *a*, *b*, *c* and *d* for this locus. Considerable variation in banding pattern was observed among the 22 cultivars and 28 taxa examined (Tables 3 and 4). In addition to the zone

Table 3 Isozyme phenotypes of 22 *Actinidia* cultivars

Cultivar	Species†	ACP-2	EST	PGI-2	PGM-2	PRX-1	PRX-2	PRX-3	PRX-4	PRX-5	TPI
Sanxia no. 1	de	BC	C	AC	BCDE	A	BC	AB	BC	A	BC
Wuzhi no. 1	ch	BC	C	ABD	BCD	AB	AB	AB	A	ABC	ABC
Daguohongxin	de	AB	C	BD	BCD	AB	B	A	BC	AC	ABC
Jianmmia no. 1	de	BC	BC	BC	BCD	A	B	A	C	C	BC
79-1	ch	B	C	ABCE	BCD	A	AB	A	AB	C	BCD
Tongshan no. 5	ch	B	C	ABCD	DE	A	AB	A	B	AC	BC
Moshan no. 7	ch	BC	BC	BCDE	BC	A	A	A	B	AC	BC
Xuxiang	de	BC	C	ACE	BCD	AB	AB	A	BC	ABC	BC
Wuzhi no. 5	ch	BC	BC	ABCD	BD	AB	B	A	B	AC	ABCD
Wuzhi no. 3	ch	BC	BC	CDE	BD	AB	ABC	A	ABC	ABC	ABC
Jianhong no. 1	de	B	B	BCDE	B	A	AB	A	C	C	BC
Sanxia (male)	de	BC	BC	AB	BCD	AB	AB	A	BC	AC	BCD
Miliang	de	BC	BC	AB	BCD	AB	B	AB	BC	AC	BD
Guihai no. 4	ch	AB	C	BC	BC	A	AB	A	C	AC	AC
Chengyang no. 4	ch	B	BC	AC	BD	AB	B	A	BC	C	AC
Qinmei	de	ABC	BC	BCDE	BCD	ABC	ABC	AB	C	C	ABC
Wuzhi no. 6	ch	BCD	BC	CD	BCD	AB	B	A	BC	AC	ABCD
Qingyuanqiucui	ch	?‡	?	AD	BC	A	AB	A	C	A	?
Wuzhi no. 2	ch	B	BC	AB	BCD	A	AB	A	C	ABC	BCD
Changan no. 4	de	B	B	AC	BCDE	B	B	A	C	ABC	BC
Hayward	de	BC	BC	BCD	BCDE	AB	B	A	C	BC	BC
81-24	ch	B	BC	ABE	BCD	AB	B	A	BC	ABC	BC

†de, *A. deliciosa*; ch, *A. chinensis*.
‡Undecided phenotype.

Table 4 Isozyme phenotypes of 28 taxa of *Actinidia*

Taxa	ACP-2	EST	PGI-2	PGM-2	PRX-1	PRX-2	PRX-3	PRX-4	PRX-5	TPI	Polyploidy†
Sect. *Leiocarpae*											
A. arguta var. *arguta*	BCD	CD	BCD	AB	AB	B	B	C	AC	AC	cyto. 2x, 4x, 6x
A. macrosperma var. *macrosperma*	ABC	BC	ABD	EFG	A	B	C BCD	B BC	C	ABC	cyto. 4x
A. macrosperma var. *mumoides*	AB?	C	BCD	AEFG	AB	B	AB	C	C	A	poly-
A. melanandra var. *melanandra*	ABC	CD D	ABCD	AR4‡C	A	B ABC	BCD AB	C CD	C AC	B ABC	cyto. 4x
A. polygama	A	E	BCD	B	AB	B	B	B	AC	ABC	cyto. 2x, 4x
A. valvata var. *valvata*	AB	CE	BC	BCEF	AB	ABCD ACD	ABCD	BCD	C BC	ABC	cyto. 4x
Sect. *Maculatae*											
A. callosa var. *discolor*	C BC	AB BCDE	CE BD	R1R2A AC	A AB	AC B	AB	BC ABC	BC ABD	BC	cyto. 4x
A. callosa var. *henryi*	ABC	B	RCD	AB	A AB	AB	B AB	B BC	C	B ABC	cyto. 2x, 4x
A. chrysantha	ABC ABCD	BC C	DE	R4C	A AB	B BC	BC	B C	AC BC	AB BC	cyto. 4x
A. cylindrica var. *reticulata*	BC	BD	CD	B	AB	B	AB	B	B	ABC	poly-
A. fasciculoides var. *fasciculoides*	AB	C	CE	B	A	?	B	C	BC	BC	
A. rufa	ABC	B BC	BCDE CDE	B	A	AB B	BC	C BC	B BC	ABC	poly-
A. rubricaulis var. *carica*	AB BC	BC	BD	R3A	AB	AB	AB	B BC	BC	A BC	
A. sabiae folia	BC	BC	AB	CD	A	B	BC	C	BC	BC	cyto. 2x
Sect. *Stellatae*											
A. chinensis var. *chinensis*	B AB BC C BCD	BC C	ABCE ABD AB ABCD BCDE CDE AC CD AD ABE	BCD DE BC BD	A AB	A AB B ABC	A	A AB B BC ABC C	A AC ABC C	AC ABC BC BCD ABCD	cyto. 2x, 4x
A. deliciosa var. *deliciosa*	AB B ABC BC C	BC C	AC BD BC ACE BCDE AB BCD	BCDE BCD B	A AB ABC B	AB B BC ABC	A AB	BC C	A AC ABC C BC	BC ABC BCD BD	cyto. 6x
A. deliciosa var. *chlorocarpa*	BC	C	ABCD	ABC	B	BC	B	C	C	ABC	poly-
A. eriantha var. *eriantha*	C	BC C	CD	B	A AB	B	BC BD	BC C	C BC	BC	cyto. 2x
A. eriantha f. *alba*	C	C	CD	B	A	AB	AB	BC	BC	AC	cyto. 2x
A. fulvicoma var. *fulvicoma*	BC	BCD	CD	BC	AB	A	BCD	BC	ABC	ABC	poly-
A. grandiflora	BC	ABC C	BC DE	C	A AB	AB B	AB BD	A BC	C	ABC	poly-
A. guilinensis	BC	C	BD	CD	A	B	B	C	BC	BC	
A. hubeiensis		B	CD	AC	ABC	B	ABC	BCD	C	ABC	poly-
A. latifolia var. *latifolia*	ABCD	C	ABCD AF	A	A AB	B AC	A B	BC C	AB C	AC	poly-
A. liangguangensis	ABC	C	ACD	ACD	A	BC	A	C	BC	ABC	poly-
A. lijiangensis		B	RABD	ABC	AB	BC	AC	C	C	BC	poly-
A. styracifolia	AB	BC	BC	BD	A	B	A	A	C	AB	
A. zhejiangensis	B	ABC	CD	R3B	A	C	AC	BC	C	ABC	poly-

†Cyto., ploidy has been previously determined by cytogenetic study; poly-, polyploidy was revealed by allozymes only in this study.
‡Irregularly spaced position of bands designated as putative rare alleles starting at number 1 for the most anodal one.

normally found in *A. deliciosa* and *A. chinensis*, two zones of enzyme activity, one anodal and one cathodal to the zone discussed above, were observed among taxa. As the genetic control of most enzymes is well enough known to allow genetic inference to be made from gel-banding patterns (Murphy *et al.*, 1990), single- or double-banded phenotypes detected in these two zones allow us tentatively to designate two additional monomeric loci, *Acp-1* and *Acp-3*, in *Actinidia* species. Most species possess either one or two ACP loci. However, it is interesting to note that *A. melanandra* Franch. (Sect. *Leiocarpae* Dunn) and *A. callosa* var. *henryi* Maxim. (Sect. *Maculatae*) possess all three ACP loci. *Actinidia melanandra* and *A. callosa* are the most widespread and presumptive progenitor species in the Sect. *Leiocarpae* and Sect. *Maculatae*, respectively (Cui, 1993).

Peroxidase (PRX)

Five zones of activity were observed for PRX (Fig. 1). In the JM family, segregation data for PRX-1, PRX-3 and PRX-4 confirmed the expected Mendelian ratios. This suggests a disomic mode of inheritance for these isozymes specified by three single genes (*Prx-1*, *Prx-3* and *Prx-4*) (Table 1). However, the expected phenotypic ratios for tetrasomic inheritance were calculated and consistent with the assumption of a Mendelian mode of tetrasomic inheritance of five genes in the WE family (Table 2). The results showed the existence of two alleles at *Prx-1* and *Prx-3*, and three alleles at *Prx-2*, *Prx-4* and *Prx-5*. Of the 22 cultivars examined, 55 per cent of the cultivars were heterozygous at *Prx-1* and *Prx-2*. A total of 18 per cent, 45 per cent and 68 per cent were heterozygous at *Prx-3*, *Prx-4* and *Prx-5*, respectively (Table 3). Altogether, 46 per cent, 39 per cent, 63 per cent, 39 per cent and 52 per cent of the plants of 28 taxa were heterozygous at *Prx-1*, *Prx-2*, *Prx-3*, *Prx-4* and *Prx-5* respectively (Table 4). Two additional zones of activity between PRX-3 and PRX-4 were observed among different species, which can be considered as species-specific rare isozymes. The zone cathodal to PRX-3 was detected in *A. sabiae*, *A. latifolia*, *A. fasciculoides* var. *fasciculoides* and *A. deliciosa* var. *chlorocarpa*. The zone anodal to PRX-4 was detected in *A. callosa* var. *discolor*, *A. cylindrica* var. *reticulata*, *A. rubricaulis* var. *carica*, *A. hubeinensis*, *A. lijiangensis*, *A. fulvicoma*, *A. grandiflora*, *A. arguta* and *A. callosa* var. *henryi*. The species of *A. zhejiangensis*, *A. eriantha*, *A. eriantha* f. *alba*, *A. chrysantha* and *A. styracifolia* exhibit both zones of isozyme activity. These rare isozymes appear to be observed mostly in species of the Sect. *Maculatae*.

Phosphoglucoisomerase (PGI)

PGI zymograms exhibited three zones of activity in *Actinidia*. PGI-1 was monomorphic in all *Actinidia* plants surveyed. PGI-3 was polymorphic, but poorly stained. This locus was subsequently excluded from further discussion. PGI-2 was polymorphic and well resolved: up to seven bands could be seen in individual plants carrying four alleles (tetra-allelic genotype). Triple-banded and five-banded phenotypes expressed by diallelic and triallelic genotypes could be reliably scored in the progenies of the crosses (Fig. 1). The observed ratios of progeny phenotypes were consistent with the expected phenotypic ratios for disomic inheritance in the JM (Table 1) and tetrasomic inheritance in the WE (Table 2) families. Five alleles, *a*, *b*, *c*, *d* and *e*, could be assigned to the *Pgi-2* locus. Of the 22 cultivars, 23 per cent, 27 per cent and 50 per cent were tri-, tetra- and diallelic heterozygotes, respectively. None of the cultivars and taxa examined was found to be homozygous for any single allele of *Pgi-2* (Tables 3 and 4). These results are in agreement with the PGI phenotypes of triple-, five- and seven-banded patterns of *A. deliciosa* cultivars reported by Messina *et al.* (1991). In all, 55 per cent, 27 per cent and 18 per cent of the plants of the 28 taxa were di-, tri- and tetra-allelic heterozygotes (Table 4). A band anodal to allele *a* was found only in *A. callosa* var. *henryi* and designated putatively as a rare allele (R).

Phosphoglucomutase (PGM)

Two zones with PGM activity were observed in *Actinidia* species. PGM-1 was monomorphic in all plants of this study. PGM-2 was polymorphic with single-, double- and triple-banded patterns. Segregation data for the JM and WE families, in which the expected phenotypic ratios were tested for disomic and tetrasomic inheritance, respectively, confirmed the presence of three alleles, *b*, *c* and *d*, at *Pgm-2* (Tables 1 and 2). One regularly spaced band anodal to the position of the *b* allele and two bands cathodal to the position of the *d* allele were found in plants surveyed among 28 taxa and were designated putative alleles *a*, *e* and *f*. Four additional bands were occasionally observed among taxa and were designated rare alleles R1–R4. Of the cultivars examined, 14 per cent, 32 per cent and 50 per cent were tetra-, di- and triallelic heterozygotes, respectively. Only 4 per cent of the cultivars were homo-

zygous at the *b* allele (Table 3). Among 28 taxa, 4 per cent of the plants were homozygous for the *a* and *c* alleles; 21 per cent were homozygous for the *b* allele (Table 4). Alleles *f* and *g* appeared to be unique alleles in the Sect. *Leiocarpae*. Alleles R1–4 were limited to the Sect. *Maculatae*, with the exception of R3, in *A. zhejiangensis* and R4 in *A. melanandra*.

Triosephosphate isomerase (TPI)

One polymorphic TPI zone was observed in *Actinidia* species (Fig. 1). Regular segregation for tetrasomic inheritance was detected at the *a*, *b* and *c* alleles of this *Tpi* locus in the WE family (Table 2). Alleles *b* and *c* were also found to segregate normally in a 1:2:1 disomic mode of inheritance in the JM family (Table 1). A significantly high level of heterozygosity was observed among cultivars, as no homozygotes were found for any single allele. Allele *b* is apparently predominant at the TPI locus. A total of 86 per cent of the cultivars were heterozygous, but had the *b* allele in common (Table 3). Some 55 per cent of the cultivars were diallelic genotypes, 32 per cent and 9 per cent were tri- and tetra-allelic genotypes, respectively. Only 5 per cent of the plants of the 28 taxa were found to be homozygous for the *a* allele, whereas 4 per cent were homozygous for the *b* allele and 56 per cent and 29 per cent of the plants were tri- and diallelic heterozygotes, respectively. Tetra-allelic heterozygotes of *Tpi* were rare in natural *Actinidia* populations.

Determining ploidy level

Although kiwifruit (*A. deliciosa*) has often been used as an example of the successful development of a worldwide fruit industry from a wild species in this century, definitive evidence is lacking as to whether *A. deliciosa* is an allo- or autohexaploid. Another commercialized species, *A. chinensis*, has both diploid and tetraploid races (Yan *et al.*, 1994), but the origin of the tetraploid, *A. chinensis*, remains unclear. Cytogenetic studies determining the ploidy level have been hindered by the very small size and numerous number of chromosomes in *Actinidia* species (Ferguson, 1990). Allelic segregation analysis is the most definitive method of distinguishing auto- from alloploidy (Krebs & Hancock, 1989). The allelic segregation observed at 10 isozyme loci presented above provides convincing evidence that *A. deliciosa* is an allohexaploid species, whereas the tetraploid, *A. chinensis*, is an autotetraploid that apparently originated from its diploid ancestor of the same species. It is interesting to note that a high percentage of selected cultivars are tetraploids. Ten of 12 *A. chinensis* cultivars appear to be tetraploid, because triallelic or tetra-allelic phenotypes were found for at least one locus (Table 3). This is much higher than is usually found in natural populations (Yan *et al.*, 1994). Further studies are needed to determine whether or not the polyploid nature is related to some desirable traits in *A. chinensis*. Polyploidy has been studied in about a dozen species of *Actinidia* (Table 4) (Xiong *et al.*, 1985; McNeilage & Considine, 1989). Allozyme analysis provides an inexpensive and efficient alternative way of detecting polyploidy when isozyme genetics become known in *Actinidia*. The results presented in Table 4 show that allozyme analysis for the detection of polyploidy was consistent with results obtained from cytogenetic studies. Ten additional species, which have not been studied for ploidy level, were revealed as polyploids by allozyme phenotypes in this study (Table 4). However, further confirmation from cytological studies is needed. Allelic segregation analysis has also been demonstrated effectively in distinguishing auto- from allopolyploidy in other plant species (Soltis & Soltis, 1987; Krebs & Hancock, 1989).

Ten isozyme loci verified in this study have provided useful markers for the further study of genetics and marker-aided selection in breeding programmes of commercial *Actinidia* species. Allozyme phenotyping should be a feasible technique for cultivar identification, because the polyploid nature of most *Actinidia* species precludes allozyme genotyping for cultivar identification. Significant isozymic variation was observed in plants of *Actinidia*, particularly in cultivars. All cultivars can be uniquely identified by any combination of three loci. The high percentage of di-, tri- or tetra-allelism found in allohexaploid *A. deliciosa* and autotetraploid *A. chinensis* indicated an effective maintenance of heterozygosity through polyploidy in *Actinidia*. As gene frequencies cannot be calculated from polyploids in natural populations even when using codominant allozymes, studies of phylogenetic relationships between species, although necessary, could be very difficult. Alternatively, allozyme phenotype frequencies might be used, although this can result in biased estimates of gene frequencies (for example, the tetraploid BC phenotype could have a *bbbc*, *bbcc* or *bccc* genotype) and needs careful consideration.

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