Use of Isozyme Polymorphism for Gender Evaluation in Kiwifruit (*Actinidia deliciosa* var. *deliciosa*)

Poonam Shirkot*, DR Sharma and CK Shirkot+**

Department of Biotechnology, +Department of Basic Sciences, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan-173230, Himachal Pradesh

This study demonstrates the usefulness and applicability of isozyme variation as sex markers for identification of staminate and pistillate cultivars of the dioecious kiwifruit (*Actinidia deliciosa* var. *deliciosa*). Seven cultivars of kiwifruit were surveyed for isozyme polymorphism by polyacrylamide gel electrophoresis using 11 enzyme systems. But only two enzyme systems peroxidase and catalase showed polymorphism leading to gender differentiation. The availability of enzyme markers for gender identification may be useful in kiwifruit breeding programmes and orchard plantations.

Key Words: Dioecious, Isozyme, Kiwifruit (*Actinidia deliciosa* var. *deliciosa*), Sex Markers

Kiwifruit (*Actinidia deliciosa* var. *deliciosa*), is a berry fruit crop indigenous to Northern and Central China and was commercially domesticated in New Zealand. (Ferguson and Eiseman, 1983). It is a perennial vine belonging to family Dilliniaceae and its dioecious nature provides a challenge for genetic and breeding research. McNeilage (1997) reported that *A. deliciosa* is hexaploid with 2n = 6x = 174. The pistillate plants are XXXXXX whereas staminates are XXXXY. Due to their small size, the X and Y chromosomes are not morphologically distinguishable and this single Y system allows the retention of dioecism.

Staminate and pistillate plants of some members of the genus *Actinidia* differ in vegetative characters (Li, 1952), but such differences are large and inconsistent. It would be an advantage to be able to determine the sex of seedlings without having to wait for 4 or 5 years before they flower. It may be possible to use biochemical tests in the form of isozyme for sex determination (Hirsch *et al.*, 1997; Deng *et al.*, 1982; Messina *et al.*, 1991).

Protein and isozyme patterns offer a quick and reliable system for identification of cultivars and are being used as fingerprints of genetic changes (Weeden and Lamb, 1985). Many authors have reported use of isozymes for verification of fruit cultivars at the nursery level. (Torres and Bergh, 1980; Hirai *et al.*, 1986; Weeden *et al.*, 1988; Sui *et al.*, 1995; Sanchez *et al.*, 1998). But there have been few attempts to identify kiwifruit cultivars as well as to study the sex determination by means of enzyme analysis (Messina *et al.*, 1991; Hirsch *et al.*, 1997).

** *Corresponding author*

Materials and Methods

The seven cultivars of *Actinidia deliciosa* var. *deliciosa* available in India and their source, used in the present study, are shown in Table 1. The material was obtained from the experimental orchard at Department of Pomology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan. Two staminate and five pistillate cultivars were used for the study.

Table 1. Name of cultivars, source and year of introduction in India

Name of Cultivar	EC No.	Year	Source
Allison (Male)	EC-24673	1963	USA
Allison (Female)	EC-24672	1967	USA
Bruno (Female)	EC-64090	1969	New Zealand
Hayward (Female)	EC-64093	1969	New Zealand
Abbott (Female)	EC-64094	1969	New Zealand
Tomuri (Male)	EC-64092	1969	New Zealand
Monty (Female)	EC-137263	1978	New Zealand

Source: Pandey and Joshi (1997)

Fresh swollen buds were excised from the seven cultivars of kiwifruit in the month of March-April and used immediately for extraction of soluble proteins for isozyme analysis as reported by Messina *et al.* (1991). Preliminary studies had eliminated young leaves as possible sample source due to poor resolution of isozymes and inconsistent staining. Only the stable, repeatable bands in each cultivar were scored. Enzyme extraction each methods used were as described earlier (Messina *et al.*, 1991). The samples were used immediately or stored at –4 °C for electrophoresis later on. For most enzymes, storage up to only 72 h was found to give

good resolution. Phosphoglucomutase (PGM) and phosphoglucoisomerase (PGI), however, were always analyzed in freshly prepared samples.

The 11 enzyme systems used in this study were resolved on four different gel buffer systems. The first buffer system was lithium borate. Tris citrate, a modification of Ashton and Braden's system (1961) comprising 0.04 M lithium hydroxide and 0.19 M boric acid, pH : 8.3 as an electrode buffer. The gel buffer consisted of 0.05 M Tris, 7 mM citric acid (pH : 8.3). This system was used for aspartate amino transferase (AAT) and phosphoglucomutase (PGM). Alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), catalase (CAT), peoxidase (PER), Esterase (EST) and phosphoglucoisomerase (PGI) were resolved using the continuous Tris borate buffer pH : 8.2 as modified by Poulik (1957). The electrode buffer contained 0.3 M boric acid and 0.06 M sodium hydroxide pH : 8.2 and gel buffer had 0.08 M Tris and 7 mM citric acid. Whereas, acid phosphatase (ACP) and alkaline phosphatase (ALP) and phosphorylase (PR) were resolved by a third system-Tris citrate buffer pH : 7.0 as modified by Meizel and Markert (1967) having 0.05 mM Tris and 0.016 M Citric acid as an electrode buffer and 0.017 M Tris and 0.005 M Citric acid in gel buffer.

Staining recepies for ADH, AAT, ALP and ACP were used as described by Shaw and Prasad (1970). Staining of PGI, PGM and MDH was done according to Soltis *et al.*. 1983 while the methods of Conkle *et al.*, (1981) were employed for EST and CAT. Staining procedures of Graham *et al.* (1964) were employed for PER and PR.

Polyàcrylamide gel electrophoresis was carried out to separate various isozymes due to its high resolving power, transparency and chemical inertness. Anionic system (Ornstein, 1964; Davis, 1964) was used for different isozyme systems.

Differences in enzyme banding patterns (phenotypes) were recorded as differences in relative mobility, the migration of each band relative to the protein front.

For statistical analysis, bands across all enzyme systems and entries were coded as present (1) or absent (0). Genetic similarities were calculated based on the method of Sneath and Sokal (1973) and relationship between entries were quantified using the complementary similarity indices. A dendrogram was constructed based on the genetic distance matrix data by applying the unweighted pair group method with arithmetic averages (UPGMA) using the numerical taxonomy and multivariate analysis system computer program (NTSYS-PC version 1.8).

Results and Discussion

Variability in banding patterns for differentiation of gender was observed only in two of the 11 enzyme systems used. The enzymes ADH, ALP, ACP, PGM and PR, showed identical banding patterns in all the cultivars and six enzyme systems *i.e.* MDH, PER, CAT, AAT, PGM and EST showed polymorphism. Sex-related polymorphism was depicted only by PER and CAT. On the basis of their banding patterns, of PER and CAT, the cultivars were divided into two classes of staminate and pistillate cultivars.

In peroxidase system four zones of activity appeared (Fig. 1). The fastest migrating one was monomorphic and showed a single band at RM 0.9 from the origin followed by another monomorphic band at RM 0.7. The male and female phenotypes could be distinguished through the presence or absence of a band at RM 0.60. It was found only in five female cultivars and absent in the male cultivars. Another monomorphic band was found in the anodal region at position RM 0.04.

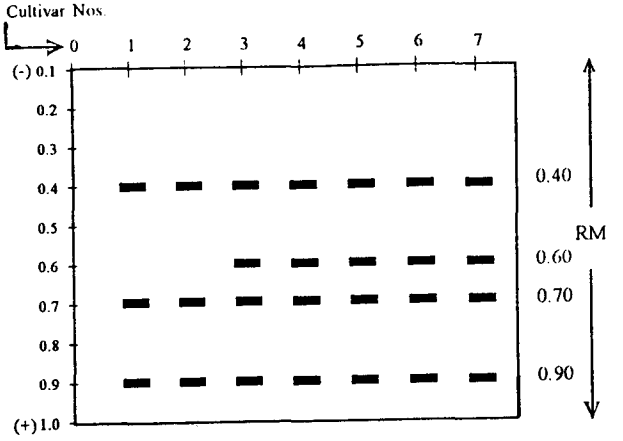

Fig. 1. Isozymic pattern of peroxidase (PER) enzyme (EC 1.11.1.7) in seven cultivars of Actinidia deliciosa var. deliciosa plant

Cultivars: 1. Tomuri male 2. Allison male 3. Hayward female 4. Bruno female 5. Monty female 6. Allison female 7. Abbot female

In the catalase isozyme system, four bands were distributed between positions RM 0.15 to 0.85 (Fig. 2). The perusal of the data revealed two types of banding patterns with bands at different positions. The first banding pattern was observed only in two male

cultivars, whereas, second banding pattern was specific to the five female cultivars. First band at position RM 0.85 was present in both male cultivars and absent in all the five female cultivars, whereas, second band at RM 0.75 was present only in the female cultivars. Third and fourth bands at positions RM 0.60 and 0.15 were monomorphic and overlapped in all the seven cultivars.

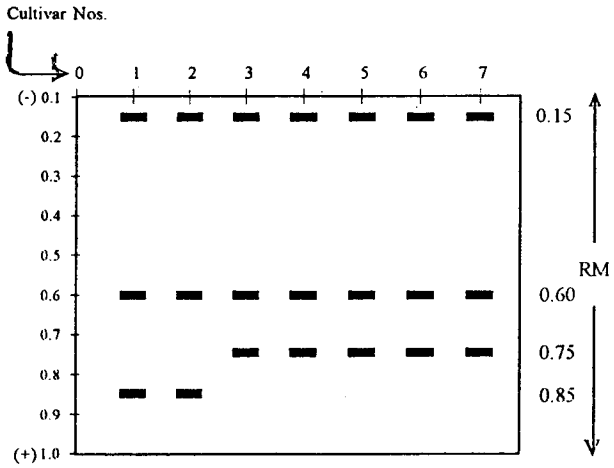

Fig. 2. Isozymic pattern of Catalase (CAT) enzyme (EC 1.11.1.6) in seven cultivars of Actinidia deliciosa *var.* deliciosa *plant*

Genetic similarity coefficients derived from pair-wise comparison among the pistillate and staminate cultivars are summarized in Fig. 3. The index value ranged from 0.73 to 0.88 with the mean of 0.81. The dendrogram showed two clusters of male and female cultivars. The cuitivar Allison (male) was found to be closer in distance to Tomuri than to any other cultivar. This indicates a high level of polymorphism, which is expected since the genus *Actinidia* is dioecious.

To our knowledge, not much genetic data based on biochemical markers are yet available on kiwifruit. However, Hirsch *et al.* (1997) have developed a peroxidase test for sex screening in young kiwifruit seedling. In their opinion, peroxidase may be useful to determine the sex of kiwifruit because this enzyme system is involved in the hormonal sex control in dioecious plants. Thus, present data are useful because the feasibility of such an analysis in this species is demonstrated. The availability of this material is particularly important for our study because in a dioecious species like *A. deliciosa* var. *deliciosa* individuals are expected to be highly heterozygous and this introduces an element of uncertainty in the cultivars. The present study shows distinction between male and female cultivars with respect to

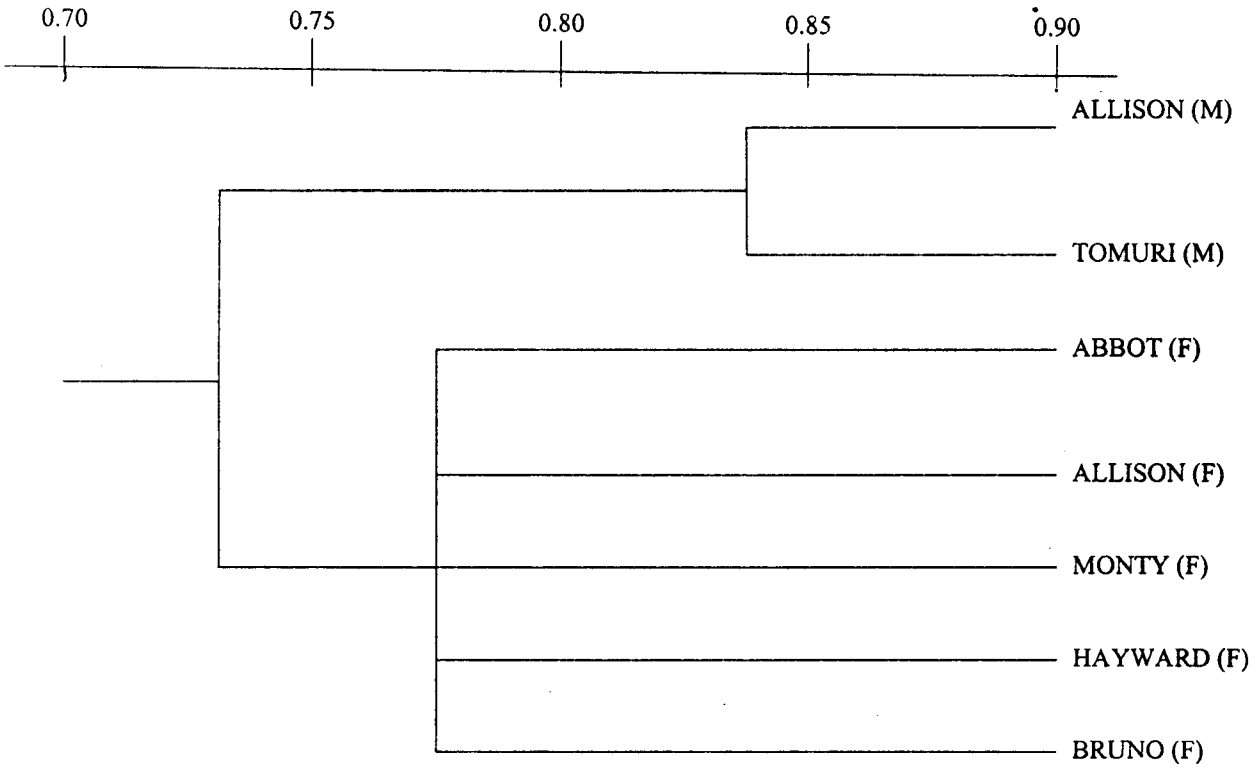

Fig. 3. Genetic relatedness among seven cultivars of Actinidia deliciosa var. deliciosa based on Peroxidase (PER) and Catalase (CAT) isozyme systems

peroxidase and catalase isozyme patterns. Catalases have so far not been used for sex determination of male and female seedlings. One band of peroxidase with RM 0.60 was observed only in five female cultivars (Fig. 1) suggested its female-associated nature. Also, one band of catalase with RM 0.85 consistently appeared only in male genotype (Fig. 2) suggesting thereby the male-associated nature of this marker.

References

Ashton GC and AWH Braden (1961) Serum B-globulin polymorphism in mice. *Australian J. Biol. Sci.* **14**: 248-254.

Conkle MT (1981) Isozyme variation and linkage in some conifer species. In: Conkle MT (ed.) *Proc. Symp. Isozymes.* North Am. Forest Trees and Forest Insects, USDA Forest Serr. Pacific SW Forest and Range Expl. Sta. Berkley, California, *Gen. Tech. Rep.* PSW, 48p.

Davies J (1964) Disc electrophoresis II. Methods and application of human serum proteins. *Ann. N. Y. Acad. Sci.* **121**: 404-427.

Deng Y, X Shangguan, Z Zhou and J Cao (1982) A preliminary report of studies on identification of the female and male vines of *Actinidia chinensis* Planch. *Acta Hortic. Sin.* **9**: 63-66.

Ferguson AR and A Eiseman (1983) Estimated annual removal of macro-nutrients in fruit and pruning from a kiwifruit orchard. *New Zealand J. Agric. Res.* **26**: 115-117.

Graham RC, U Lundholm, MJ Karnovsky (1964) Cytochemical demonstration of peroxidase activity with 3-amino-9-ethyl carbazole. *J. Histochem. Cytochem.* **13**: 150-152.

Hirai MZ, I Kozaki and I Kajiura (1986) Isozyme analysis and phylogenetic relationships of citrus. *Jpn. J. Breed.* **36**: 377-389.

Hirsch AM, D Fortune, L Chalak, JM Legave, J Chat and R Monet (1997) Peroxidase test: A test for sex screening in kiwifruit seedlings. *Acta Hortic.* **444**: 89-95.

Li HL (1952) A taxonomic review of the genus *Actinidia chinensis* Planch. *Acta Phytotaxon Sin.* **20:** 101-104.

McNeilage MA (1997) Progress in breeding hermaphrodite kiwifruit cultivars and understanding the genetics of sex determination. *Acta Hortic.* **444**: 72-78.

Meizel S and CL Markert (1967) Malate dehydrogenase isozymes of the Marine Snail *Ilianassa obsoleta. Arch. Biochem. Biophys.* **122**: 753-756.

Messina R, R Testolin and M Morgante (1991) Isozymes for cultivars identification of kiwifruit. *Acta Hortic.* **297**: 63-69.

Ornstein L (1964) Disc electrophoresis I. Background and theory *Ann. N.Y. Acad. Sci.* **121**: 321-349.

Pandey G and BD Joshi (1992) *Kiwifruit: A Future Fruit for Indian Hills*, NBPGR, New Delhi, p17.

Poulik MP (1957) Starch gel electrophoresis in a discontinuous system of buffers. *Nature* **180:** 1477-1479.

Sanchez EE, JM Ortiz and JL Cenis (1998) Identification of table grape cultivars (*Vitis vitifera* L.) by the isozymes from woody stems. *Genet. Resour. Crop Evol.* **45:** 173-179.

Shaw CR and R Prasad (1970) Starch gel electrophoresis of enzymes – a compilation of reciepies. *Biochem. Genet.*, **4:** 297-320.

Sneath PHA and RR Sokal (1973) *Numerical Taxonomy.* Freeman. San Fransisco.

Soltis DE, CH Haufler, DC Darrow and GJ Gastony (1983) Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers and staining schedules. *Am. Fern. J.* **73**: 9-27.

Sui LF, RM Zee, M Manshardt, MK Aradhaya (1995) Enzyme polymorphism in *Cananium. Sci. Hort.* **68**: 197-206.

Torres AM and BO Bergh (1980) Fruit and leaf isozymes as genetic markers in avocado. *J. Am. Soc. Hortic. Sci.* **105**: 614-619.

Weeden NF and RC Lamb (1985) Identification of apple cultivars by isozyme phenotypes. *J. Am. Soc. Hortic. Sci.* **110**: 509-515.

Weeden NF, BI Reisch and MHE Martens (1988) Genetic analysis of isozyme polymorphism in grape. *J. Am. Soc. Hortic. Sci.* **113**: 765-769.