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Characterization of two interspecific hybrids of *Piper*

By B. SASIKUMAR*, B. CHEMPAKAM, JOHNSON K. GEORGE, A. B. REMASHREE, S. DEVASAHAYAM, K. P. M. DHAMAYANTHI, P. N. RAVINDRAN and K. V. PETER
Indian Institute of Spices Research, Marikunnu P.O., Calicut 673012, Kerala, India
(e-mail: iisrspices@x400.nicgw.nic.in)

SUMMARY
Two interspecific hybrids of *Piper*, *P. nigrum* x *P. attenuatum* and *P. nigrum* x *P. barberi*, produced for the first time, were characterized by morphology, anatomy, isozymes, cytology and function (reaction to *pollu* beetle). The hybrids exhibit distinct morphological and anatomical features. Hybrid-specific bands as well as male-specific bands were observed in the zymograms of the isoforms of three of the four isozymes, peroxidase, esterase and polyphenol oxidase. Paired affinity index of the four enzymes revealed more similarity between the female parents and hybrids than between the male parents and hybrids. The hybrids had the same chromosome number (2n = 52) as their parents. The leaves of the hybrids were less preferred for feeding by *pollu* beetles when compared with their female parents. Successful hybridization among the three species belonging to the same subgenus *Maricha* confirms their phylogenic relationship.

Black pepper (*Piper nigrum* L.) is a perennial climber from which the black pepper of commerce is obtained. The genus *Piper* (Piperaceae) is represented by 17 species in South India. Studies on South Indian *Piper* species are limited to species enumeration and floristics (Hooker, 1886; Gamble, 1925; Rahiman and Nair, 1987) in addition to anatomy (Murty, 1959; Pal, 1961; Menancherry, 1993), chemotaxonomy and numerical taxonomy (Ravindran, 1990).

Although India is a leading producer of black pepper in the world, the productivity of the crop is low due to various factors among which infestation by *pollu* beetle (*Longitarsus nigripennis* Mots.) has been identified as a major factor. The pest damage accounts for up to 30–40% losses in yield in endemic areas (Devasahayam *et al.*, 1988). At present, chemical control measures using insecticides are practised to manage the pest. However, the increasing demands by importing countries for "clean spices" free from pesticide residues, have necessitated the need for breeding resistant varieties. A higher degree of stable resistance was observed in related species such as *P. attenuatum* and *P. barberi*, when compared with cultivars/varieties (IISR, 1994). *P. attenuatum* (2n = 52) is a fast-growing wild species prevalent in the Western Ghat forests and the Eastern tropical Himalayas. *P. barberi* (2n = 52), a distinct, small, shy climber is also found in Western Ghat forests.

Interspecific hybridization in South Indian *Piper* assumes significance for introgression of alien genes to cultivated black pepper, *P. nigram*, especially with regard to resistance to *pollu* beetle and *Phytophthora capsici* and also from the biosystematic angle. The present paper describes, for the first time, the production and characterization of two interspecific hybrids of *Piper*.

*Corresponding author.

MATERIALS AND METHODS

Two cultivars of *Piper nigrum* (2n = 52), Aimpiriyan and Karimunda were hybridized as female parents with *P. attenuatum* and *P. barberi*, respectively during 1992. Pollen grains collected from the male parents were repeatedly brushed (six times) on to the female spikes of 20 cultivars to ensure fertilization as there is a chronological maturity of flowers in a spike. The matured berries were harvested and sown immediately in separate sand filled basins. The hybrid seedlings were isolated, multiplied vegetatively and grown in the nursery in polybags. Hybridity of these lines was confirmed based on morphology, biochemistry, anatomy, cytology and also functionally (reaction to *pollu* beetle). All observations were recorded from cuttings of the same age.

Morphology

Records were kept of morphological characters such as leaf length, leaf width, leaf area, petiole length, internode length, shoot tip colour, leaf and leaf tip shape, leaf texture and appearance of the plant, from the parents and the putative hybrids (ten plants each).

Anatomy

Stems collected from cuttings of the putative hybrids and parents were cut into 1 cm pieces and fixed in formalin-acetic acid-alcohol mixture. The materials were processed for microtomy as per standard procedures (Johansen, 1940). Sections were cut at 5 μm thickness in an electric sledge microtome and stained with toludine blue for histological studies (Krishnamoorthy, 1988).

Biochemical characters

Isozyme profiles of the four enzymes viz., peroxidase, esterase, polyphenol oxidase and superoxide dismutase were studied. Leaf proteins from the parents and the

putative hybrids were isolated and subjected to polyacrylamide gel electrophoresis (PAGE), followed by differential staining for the isozymes. Extraction of protein was carried out at 4°C using Tris HCl buffer (0.05 M, pH 7.4) containing 0.5% mercaptoethanol, 0.1% ascorbic acid and 0.1% cystine HCl. The extracts were filtered through a double layered cheese cloth and centrifuged at 12000 **g** for 20 min at 4°C. The supernatant was used for electrophoretic studies.

The samples (50 µl) were applied to the slots in the slab gel with a 7.5% concentration of acrylamide for resolving gel and 2.5% for stacking gel. The samples were run at 70 V for the stacking gel and 150 V for the resolving gel (Hames, 1994). The reservoir buffer system used was Tris-Glycine buffer, pH 8.3. Standard staining procedures were followed for staining the gel. For esterase (EC 3.1.1.2) the method of Harris and Hopkins (1976) was adopted. Superoxide dismutase (SOD) (EC 1.15.1.1) isozymes were stained achromatically using the method of Ravindranath and Fridovich (1975). Peroxidase (EC 1.11.1.7) isozymes were observed by specific staining method of Shimoni and Reuveni (1988). Isozyme bands of polyphenol oxidase (PPO) (EC 1.14.18.1) were marked after staining by Holstein's (1967) method.

Rm (Relative electrophoretic mobility) values for each isozyme band were noted as the ratio of distance travelled by the band to the distance travelled by the marker dye. Paired affinity index (PAI) between a parent and hybrid was calculated as the number of shared bands divided by the total number of bands. The shared bands were counted irrespective of the intensity of staining as well as the thickness of bands. Sampling was done twice and electrophoresis was performed in duplicate to obtain consistency of bands.

Cytology

Actively growing root tips were collected from cuttings of the putative hybrids and the parent species between 11.00 and 12.00 hours and treated with 1% solution of α-bromonaphthalene at 4–5°C for 4 h. The materials were then washed thoroughly in distilled water and fixed in 3:1 mixture of ethanol, acetic acid and chloroform for 24 h. The fixed root tips were hydrolyzed with 1 N HCl at 60°C for 15–20 min and stained in 2:1 lactopropionic orcein for 4 h and squashed in 45% propionic acid. Five well-spread mitotic metaphase plates each from two slides were used for counting chromosome number (Nair *et al.*, 1993).

Functional evaluation

The putative hybrids and parents were evaluated functionally for their reaction to *pollu* beetle feeding. Leaf discs of 1 cm^2 were cut from the parent species and hybrids and placed over moist filter paper in 100 ml beakers. Five adult *pollu* beetles collected from the field were starved for 12 h and introduced into the beakers and covered with a muslin cloth. The area fed by the beetles was measured 24 h after release by placing the leaf discs over a grid under a stereo microscope. The experiment was replicated six times and the data were analyzed statistically.

RESULTS

Morphology

The hybrids were intermediate between the parental species for most of the morphological (metric) characters (Table I). For the characters such as shoot tip colour, leaf tip shape and leaf shape, the hybrid *P. nigrum* x *P. attenuatum* resembled the female parent, *P. nigrum*. The leaf lamina of the hybrid was wrinkled. Although *P. nigrum* x *P. barberi* inherited the shoot tip colour and leaf tip nature from the maternal parent, its leaf shape was intermediate between the parents. However, the leaf texture of this hybrid was akin to the male parent. Although *P. barberi* was very rich in mucilage, the hybrid had very little mucilage in the leaves.

Anatomy

Stem anatomy of the species and the hybrids showed both structural as well as dimensional variations. *P. nigrum* (cvs Aimpiriyan and Karimunda) had mucilage canals in the centre of the pith as well as periphery (Figure 1), whereas *P. attenuatum* had only a comparatively small mucilage canal in the centre (Figure 2). In *P. barberi*, seven mucilage canals were seen in the periphery and a very large cavity in the centre of the pith (Figure 3). The hybrid, *P. nigrum* x *P. attenuatum* was intermediate between the parents for the nature of the central mucilage canal (Figure 4). A single large

TABLE I
Morphological characters of Piper *species and species hybrids*

Species/ Hybrid	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Petiole length (cm)	Internode length (cm)	Shoot tip colour	Leaf tip	Leaf shape	Leaf texture	Remarks
P. nigrum (cv. Aimpiriyan)	11.7±1.2	7.8±1.09	74.7±9.5	5.26±1.0	8.44±0.73	purple	acuminate	cordate	glabrous membranous	green plant, without mucilage
P. attenuatum	6.72±0.68	4.51±0.72	22.6±6.1	3.02±0.23	6.35±0.55	white	acute	ovate	glabrous membranous	dark green plant, without mucilage
P. nigrum x *P. attenuatum*	7.55±0.79	5.03±0.8	24.4±4.7	3.15±0.29	4.91±0.56	purple	cuspidate	ovate	glabrous membranous	dark green plant, without mucilage
P. nigrum (cv. Karimunda)	10.8±1.0	6.45±0.78	45.7±6.0	4.2±0.43	7.38±0.85	purple	acuminate	cordate	glabrous membranous	green plant, wrinkled leaf lamina
P. barberi	13.5±0.78	5.03±0.87	40.7±7.33	0.97±0.19	3.19±0.75	white with purple spathe	acuminate	lanceolate	smooth	pale green plant, highly mucilaginous nature
P. nigrum x *P. barberi*	9.38±0.41	5.77±1.0	37.6±9.4	3.52±0.87	6.12±1.06	purple	acuminate	intermediate between cv. Aimpiriyan & *P. barberi*	smooth	pale green plant with sparse mucilage

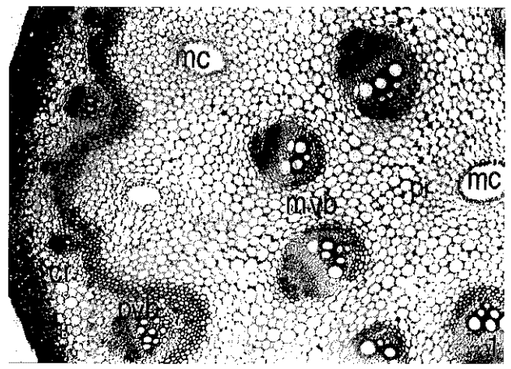

FIG. 1
T.S. of *Piper nigrum* stem showing peripheral bundles, medullary bundles and mucilage canals × 200.

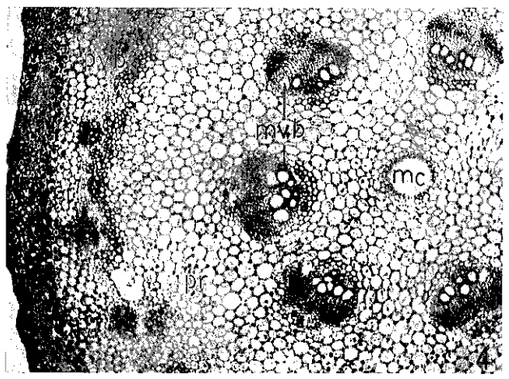

FIG. 4
T.S. of *P. nigrum* x *P. attenuatum* hybrid showing intermediate characters of the parents × 200.

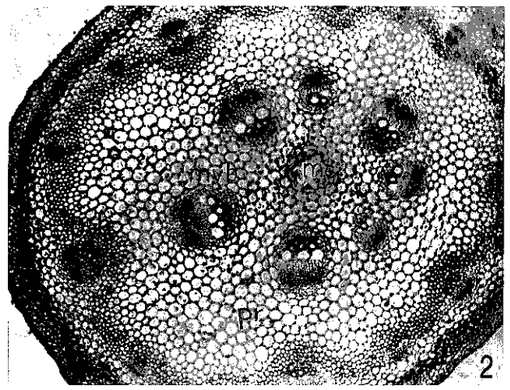

FIG. 2
T.S. of *P. attenuatum* stem with fewer bundles and mucilage canals × 200.

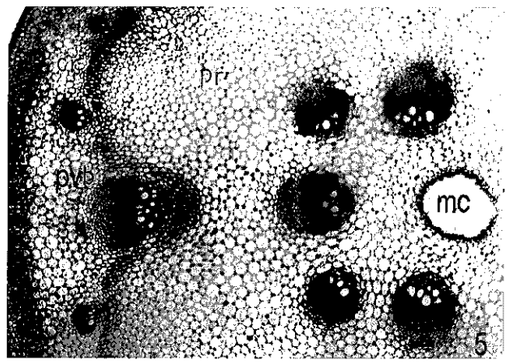

FIG. 5
T.S. of *P. nigrum* x *P. barberi* hybrid showing intermediate characters of the parents × 200.

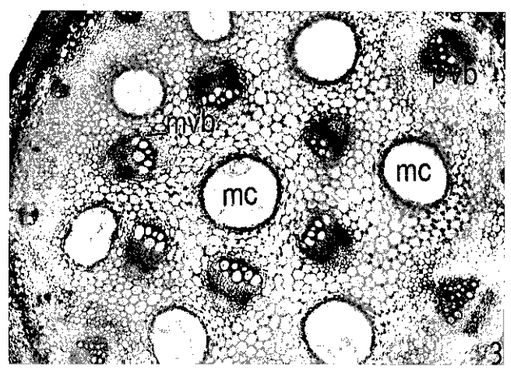

FIG. 3
T.S. of *P. barberi* stem with fewer bundles and more mucilage canals × 200.

(**cr** – cortical region; **mc** – mucilage canal; **mvb** – medullary vascular bundles; **pr** – pith region; **pvb** – peripheral vascular bundle.)

TABLE II
Dimensional variation for anatomical traits in Piper *species and species hybrids, all measurements in μm*

Species/hybrid	No. of peripheral vascular bundles	No. of medullary vascular bundles	No. of tracheids in peripheral bundles	No. of tracheids in medullary bundles	Width of tracheids in peripheral bundles	Width of tracheids in medullary bundles	Diameter of central mucilage canal	Diameter of mucilage canal in outer pith region
P. nigrum (cv. Aimpiriyan)	30±1.2	8±0	9.6±1.3	10.4±1.5	31.6±8.5	66.6± 7.4	325 ±25	159.6± 9
P. attenuatum	16±0.1	4±0	12.8±4.4	10 ±1.8	36.8±2.3	62.2± 2.2	86.7±10	Nil
P. nigrum x P. attenuatum	25±0.1	6±0	10.4±1.8	6.6±1.4	25.5±2.2	31 ± 2.6	178.4± 7.8	Nil
P. nigrum (cv. Karimunda)	26±1.4	8±0	8.1±2.4	18.6±2.8	34.8±3.4	62.5± 8.2	194.5± 5.3	71.6±15.5
P. barberi	16±0.8	7±0	15 ±2.8	13.3±3.7	26.3±2.5	36.3± 5.8	410 ± 8.8	222 ±57
P. nigrum x P. barberi	29±0.8	9±0	29±1.5	10.6±2.6	26.2±2.6	34.6±27	446 ±27	Nil

mucilage cavity was observed in the centre of the pith in the hybrid *P. nigrum* x *P. barberi* (Figure 5). There were more vascular bundles in *P. nigrum* cultivars than in the paternal species, *P. attenuatum* and *P. barberi* (in Figure 1, only one portion of the T.S. of *P. nigrum* is seen) (Table II). In the hybrid *P. nigrum* x *P. attenuatum*, the number of vascular bundles was intermediate between the parents whereas in the other hybrid, the number of vascular bundles was more or less the same as in the female parent (Figures 1–5 and Table II).

Apart from differences in structure, the species and the putative hybrids showed dimensional variations for the different anatomical traits (Table II). The hybrid *P. nigrum* x *P. attenuatum* was intermediate between the two species for the number of peripheral and medullary vascular bundles and the number of tracheids in the peripheral vascular bundles. The hybrids had fewer tracheids in the medullary bundles than the parents. Tracheids in the peripheral and medullary bundles in the hybrid were narrower than those of the parents, whereas the diameter of the mucilage canal in this hybrid was intermediate between those of the parents.

The hybrid *P. nigrum* x *P. barberi* resembled more or less closely the female parent in the number of peripheral vascular bundles and the number of medullary vascular bundles. The hybrid resembled its male parent for the number of tracheids in the peripheral bundles as well as for the width of the tracheids in the peripheral and medullary bundles.

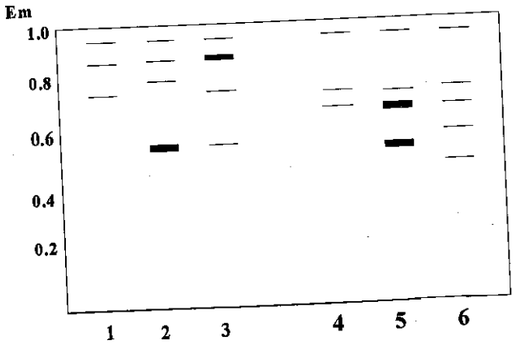

FIG. 6
Peroxidase zymogram of interspecific hybrids of *Piper* and their parents. 1 = *P. barberi*, 2 = *P. nigrum* x *P. barberi*, 3 = *P. nigrum* (Karimunda), 4 = *P. attenuatum*, 5 = *P. nigrum* x *P. attenuatum*, 6 = *P. nigrum* (Aimpiriyan).

Biochemical markers

Peroxidase zymograms of *P. attenuatum* and *P. nigrum* (cv. Aimpiriyan) were different (Figure 6), but shared three anodal isoperoxidases which were also expressed in the hybrid *P. nigrum* x *P. attenuatum* of which one band (Rm = 0.71) was intense in the hybrid (Figure 6). Further, a thick hybrid specific band (Rm = 0.54) was also observed in the interspecific hybrid. PAI estimated between the parents, *P. nigrum* and *P. attenuatum* and their hybrid revealed 67% similarity between the female parent and the hybrid and about 57% similarity between the male parent and the hybrid (Table III).

The hybrid *P. nigrum* x *P. barberi* inherited three of the four bands from the female parent *P. nigrum*, cv. Karimunda, of which one band (Rm = 0.57) appeared intense in the hybrid. The band at Rm = 0.75 was not expressed in the hybrid. The hybrid also had a hybrid-specific thin band at Rm = 0.80. Three of the anodal isoperoxidases were common to both the parent species. PAI estimated between the parental species and the hybrid revealed 75% similarity between the female parent and the hybrid, whereas that between the male parent and the hybrid was 57% (Table III).

A hybrid-specific band (Rm = 0.42) was observed for esterase isozyme in the hybrid *P. nigrum* x *P. attenuatum*. Except for this one, all other bands of the hybrids were common to the male parent, *P. attenuatum*. The female parent *P. nigrum* and the male parent *P. attenuatum* had three prominent common bands (Rm = 0.07, 0.3, 0.61) (Figure 7). The similarity between the male parent and hybrid was 72% and between the female parent and the hybrid 54%.

Esterase zymogram showed five common bands between the hybrid *P. nigrum* x *P. barberi* and the female parent, *P. nigrum*, of which three bands were also observed in the male parent *P. barberi*. Two thick unique bands specific to the male parent were also observed for this isozyme. Although no hybrid specific band was observed in the hybrid, the band at Rm = 0.08 was thicker in the hybrid than in either parent (Figure 7). About 83% affinity was noticed between the female parent and the hybrid whereas that between the male parent and the hybrid was only about 33%.

For polyphenol oxidase, the hybrid *P. nigrum* x *P. attenuatum* was characterized by two distinct bands (Rm = 0.3 and 0.88); that at Rm = 0.88 was the contribution of the female parent whereas the other band was common to both the parents (Figure 8). The hybrid showed 100% similarity between the female parent *P. nigrum* for this isozyme while the similarity

TABLE III
Percentage similarity indices for four isozymes in interspecific hybrids and their parents (Hybrid 1 = P. nigrum x P. barberi, *Hybrid 2* = P. nigrum x P. attenuatum)

Hybrid/Parent	Esterase	Peroxidase	Polyphenol oxidase	Superoxide dismutase	Total for 4 isozymes
Hybrid 1 with *P. barberi*	16.6	28.5	0.0	33.3	19.6
Hybrid 1 with *P. nigrum* (cv. Karimunda)	41.7	37.5	16.7	25.0	31.2
Hybrid 2 with *P. attenuatum*	36.3	28.6	25.0	33.3	30.8
Hybrid 2 with *P. nigrum* (cv. Aimpiriyan)	27.2	33.3	50.0	28.6	34.8

between the hybrid and male parent was only 50% (Table III). In the hybrid *P. nigrum* x *P. barberi*, three hybrid specific bands at Rm = 0.32, 0.44 and 0.85 were observed, of which the last two were thick and easily detectable. The parents shared one band in common (Rm = 0.37). The similarity between the hybrid and the female parent was 33% while there was no similarity (0%) between the male parent and the hybrid.

Only two isoforms of superoxide dismutase were observed in the hybrid *P. nigrum* x *P. attenuatum* (Rm = 0.45, 0.64). One band (Rm = 0.45) was also observed in the parent species. The band at Rm = 0.45 was very thick in the parent and hybrids as well (Figure 9). The hybrid was more similar to the male parent in this case (67%) than the female parent, *P. nigrum* (57%).

The hybrid *P. nigrum* x *P. barberi* showed three isoforms of superoxide dismutase of which two (Rm = 0.22, 0.42) were contributed by the male parent. The other band (Rm = 0.54) which was feeble in the hybrid was shared by both the parents (Figure 9). This band assumed an intense form in the male parent. More similarity was observed with the female parent (67%) than the male parent (50%).

Peroxidase showed a single hybrid specific band in both hybrids *P. nigrum* x *P. attenuatum* and *P. nigrum* x *P. barberi* (Rm = 0.54 and 0.80, respectively), while esterase exhibited a specific band only with the former (Rm = 0.42). The hybrid *P. nigrum* x *P. attenuatum* had no hybrid specific band, while *P. nigrum* x *P. barberi* had three hybrid (Rm = 0.32, 0.44 and 0.85) specific bands. Superoxide dismutase had no hybrid specific bands.

When the paired affinity indices of the four enzymes were considered together, the hybrid *P. nigrum* x *P. attenuatum* had 70% overall similarity with the female parent *P. nigrum* and 62% affinity with the male parent. Similarly, the overall similarity between the hybrid *P. nigrum* x *P. barberi* and the female parent was 62% and with the male parent 39%. Thus in both cases the hybrid was more similar to the female parent.

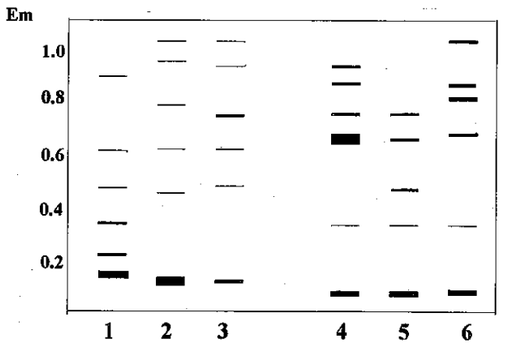

FIG. 7
Esterase zymogram of interspecific hybrids of *Piper* and their parents. 1. = *P. barberi*; 2. = *P. nigrum* x *P. barberi*; 3. = *P. nigrum* (Karimunda); 4. = *P. attenuatum*; 5. = *P. nigrum* x *P. attenuatum*; 6. = *P. nigrum* (Aimpiriyan).

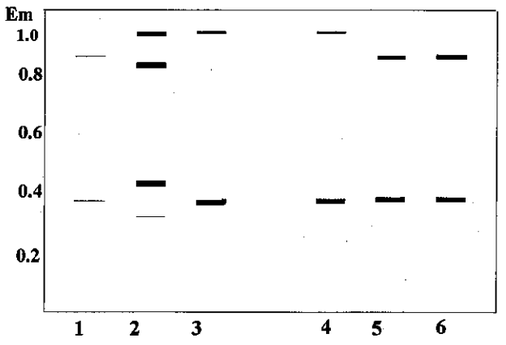

FIG. 8
PPO zymogram of interspecific hybrids of *Piper* and their parents. 1. = *P. barberi*; 2. = *P. nigrum* x *P. barberi*; 3. = *P. nigrum* (Karimunda); 4. = *P. attenuatum*; 5. = *P. nigrum* x *P. attenuatum*; 6. = *P. nigrum* (Aimpiriyan).

Cytology

The hybrids had 2n = 52 chromosome in their somatic cell as did the parent species.

Functional analysis

The hybrids and parents differed significantly in their reaction to *pollu* beetle feeding (Table IV). The leaves of the hybrids were significantly less preferred for feeding than those of their female parents.

DISCUSSION

Two hybrids of black pepper have been produced for the first time that were significantly less attractive to feeding by *pollu* beetles. Earlier attempts to cross species of *Piper* with a view to transfer resistance to *pollu* beetle or *Phytophthora* foot rot resistance were not successful (IISR, 1992). In the present study, although 20 cultivars were used as female parents, success was obtained only with cv. Aimpiriyan and cv. Karimunda, probably due to the genetic combination of the cultivar/species. Further, unlike in the earlier attempts, repeated pollination (at least six times) of the spikes of the female parent was done. Systematic interspecific hybridization involving more cultivars and repeated pollination of the spikes of the female vines are probably the major factors which helped in obtaining a few interspecific hybrids in the present study, which were multiplied further vegetatively.

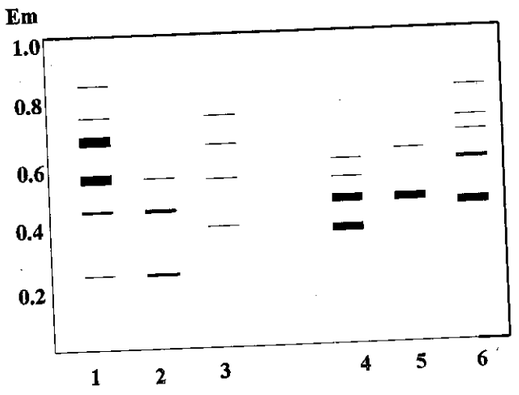

FIG. 9
SOD zymogram of interspecific hybrids of *Piper* and their parents. 1. = *P. barberi*; 2. = *P. nigrum* x *P. barberi*; 3. = *P. nigrum* (Karimunda); 4. = *P. attenuatum*; 5. = *P. nigrum* x *P. attenuatum*; 6. = *P. nigrum* (Aimpiriyan).

The low frequency of the hybrids recovered in the present study may be attributed to the failure of some of the hybrid seeds to germinate or death of the hybrid seedlings at an early stage. The interspecific hybrid *P. nigrum* x *P. attenuatum* had wrinkled leaf lamina. Pal and Khoshoo (1972) reported inviability, weakness and deformity including virus-like syndrome in the leaves of interspecific hybrids of *Amaranthus caudatus* x *A. hypochordriacus*. The evidence presented on the morphology, anatomy, isozyme, cytology and reaction to *pollu* beetle feeding by the parent species and putative hybrids established the identity of the hybrids.

Stem anatomy of *Piper* species was studied by Metcalfe and Chalk (1950), Murty (1959), Pal (1961), Ravindran (1990) and Menancherry (1993). These workers reported the presence of central and peripheral mucilage canals in *P. nigrum*, *P. attenuatum* and *P. barberi*. In the present study also, mucilage canals were seen in all the species. The hybrids were intermediate between the parent species or inclined towards the female parents in the structure of mucilage canals and the number of vascular bundles. Variation in the number and arrangement of bundles as well as the structure and position of the mucilage canals were used as distinguishing anatomical criteria for classifying *Piper* sp. (Solereder, 1908; Metcalfe and Chalk, 1950).

TABLE IV
Reaction of Piper spp. *and species hybrids to* pollu *beetle feeding*

Species/Hybrid	Leaf area fed by beetle per 24 h (mm^2)
P. nigrum (cv. Aimpiriyan)	4.05
P. attenuatum	0.11
P. nigrum x *P. attenuatum*	0.80
CD ($P = 0.05$)	0.42
CV%	7.20
P. nigrum (cv. Karimunda)	3.74
P. barberi	0.03
P. nigrum x *P. barberi*	0.46
CD ($P = 0.05$)	0.38
CV%	6.71

Isozymes have been used extensively for identifying species hybrids (Adams and Coutinho, 1976; Tobolski and Conkle, 1977; Morris *et al.*, 1980; Mallikarjuna and Moss, 1995). Hybrid specific bands have been used to identify species hybrids in *Cajanus* (Mallikarjuna and Moss, 1995), *Cassava* (Lefevre and Charier, 1993), *Lycopersicon* (Tanksley and Jones, 1981) and *Pinus* (Adams and Coutinho, 1976; Morris *et al.*, 1980), etc. The four enzyme zymograms showed a fair amount of allelic similarity among the species and hybrids. Differences were also observed in one or more loci for all of the isozymes. Hybrid-specific bands at new loci were observed for peroxidase, esterase and polyphenol oxidase. Differences were also observed for the zymograms of the four enzymes with respect to the two *P. nigrum* cultivars used in the study, Aimpiriyan and Karimunda. These two cultivars are distinct morphologically (leaf shape, leaf size, leaf area, spike length and shape and berry size) and for quality (oleoresin, essential oil and piperine). Varietal variation in isozyme profile of crop varieties are known (Bhat *et al.*, 1992; Forrest, 1994; Shamina *et al.*, 1997).

Cytologically the species had a somatic chromosome number of 2n = 52. Earlier workers also reported *P. nigrum*, *P. attenuatum* and *P. barberi* as diploid species with 2n = 52 somatic chromosomes (Mathew, 1958; Mathew and Mathew, 1952). The interspecific hybrids also had 2n = 52 chromosomes as expected.

The hybrids inherited the non-preference of *pollu* beetle for feeding from their respective resistant parents, though not completely.

P. nigrum, *P. attenuatum* and *P. barberi* are in the same subgenus *Maricha* along with a few other pendant spike bearing South Indian species (Ravindran, 1990). The banding pattern of the four isozymes in the three parent species also revealed moderate phylogenetic resemblance among these species. A common ancestry of these species, followed by introgression with different wild species, giving rise to genetically distinct species with some genes in common is a possibility, as all three species and a few other *Piper* species occur in the same locality. Speciation in the subgenus *Maricha* would not have reached the extent of complete reproductive isolation as revealed by the successful hybridization in the present study. Crossing experiments have been used to support biosystematic relationships in different species (Narasimha Rao, 1979; Pearce and Lestur, 1979). However, some authors feel that hybridization under artificial conditions to support biosystematic studies should be treated with caution (Grun, 1961; Levin, 1974).

The production of two interspecific hybrids assumes importance from the point of transferring *pollu* resistant genes from related species of *Piper* to cultivated varieties. Backcrossing followed by screening of the progenies for *pollu* resistance markers and repeated backcrossing of the lines possessing *pollu* beetle resistance with the recurrent parent will help to evolve black pepper varieties having *pollu* beetle resistance in addition to the desired agronomic/quality attributes of the recurrent parent. Such varieties are important because of the demand for "clean black pepper" free from pesticide residues.

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