Journal of Applied Horticulture, 18(3): 187-194, 2016

Molecular diversity and phytochemical characterization of *Piper* species

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Abstract

Phytochemical and molecular characterization of *Piper* species was investigated. There was a wide variation of the active compounds present in leaf and fruits of different *Piper* species/accessions. Among the two active compounds, piperine-1 content was more in *P. chaba* fruit and Piperine-2 in *P. nigrum* fruit as compared with other species. Inter Simple Sequence Repeats (ISSR) marker was also used to analyze the genetic variation between the species/accession of *Piper* species. The phylogenetic analysis generated by ISSR marker was divided into two major groups with 47% similarity. First major group is only one species (*i.e., Piper* spp. Accession -1) and also morphologically distinct from other seven species. The second group is divided into two minor groups. *Piper betle* var. Godi Balunga and *Piper betle* var. Astarangi Balunga are grouped together with 100 % similarity at genetic level, whereas, *Piper betle* var. Utkal Sudhama having 97 % similarity with *Piper betle* var. Godi Balunga and *Piper betle* var. Astarangi Balunga. Both phytochemical and molecular marker showed significant variation among and between species/accessions. This study will help for the breeding program in *Piper*.

Key words: *Piper* spp, Piperaceae, HPTLC analysis, piperine content, ISSR marker, genetic diversity analysis

Introduction

The genus *Piper* belongs to the family Piperaceae and has over 1200 species distributed widely in the tropical and subtropical regions of the world. North East part of India and Eastern Himalayas have the centres of *Piper* genetic resources. The maximum diversity is recorded in Himalayan region with diverse geology, topography and climate. The region comprises different vegetation type's along with the tropical, subtropical, temperate and alpine climatic zones. The plant grows all over India, in evergreen forests and cultivated in Assam, Tamil Nadu and Andhra Pradesh. *P. nigrum* (black pepper) is a monocious or decorous climbing vine native to Southern India and Sri Lanka and is extensively cultivated in tropical regions. The short climbing stem are very flexible with leathery blackish green leaves. *P. nigrum* and *P. betle* have grown extensively in the Western Ghats and North Eastern parts of India. *P. nigrum* known as the "King of *Piper* spices", is the most important species and widely used spices in the world.

The *Piper* species have high commercial, economical and medicinal properties and are extensively used in Indian Ayurvedic system. The *Piper longum* or pipli, is the third most economically important species of the genus *Piper* after *P. nigrum* and *P. betle*. The plant having rich sources of two major alkaloids, piperine and piperidine (Kanaki *et al*., 2008). Traditionally, the stems, roots and leaves of *P. nigrum* are used against bronchial diseases, dyspepsia, worms and amoebiosis (Ghosal *et al*., 1996). Roots and fruits of *P. chaba* have numerous applications particularly for asthma, bronchitis, fever, pain in abdomen and used as stimulant (Kirtikar and Basu,1993). The spikes have also been effectively used as an anti-inflammatory, memory enhancing and aphrodisiac agent (Mujumdar *et al*., 1999). *Piper* extracts and piperine possess inhibitory activities on prostaglandin and leukotrienes COX-1 inhibitory effect and thus exhibit anti inflammatory activity (Stohr *et al*., 2001). The fruits of *Piper longum* have also been widely used in various traditional systems of medicine. *Piper betle* has anti diabetic properties and shown a significant lowering of the blood glucose level in case of rat.

Thin-layer chromatography technique is used to separate non-volatile mixtures. Piperine is the main therapeutically active constituent of this plant. Literature survey reveals that, various chromatographic methods such as HPTLC (Suthar *et al*., 2003), HPLC (Verzele *et al*., 1999) have been reported for the quantification of piperine. This biochemical marker like active constituent gives the variation between the genotype. Apart from biochemical marker, different molecular markers have been used to identify the genotypes and generating information required for diversity analysis for conservation and management. The Inter-Simple Sequence Repeat (ISSR) markers are considered as highly informative fingerprinting tools, as no previous knowledge of the genome is required, a large number of DNA polymorphic are easily generated, they are reliable and reproducible. This marker is used successfully to analyse the genetic structure of the genotype in many species (Chawdhury *et al*., 2014; Rout *et al*., 1998; Rout, 2006). Moreover, the identification of useful traits creates a direct value for gene banks, ensuring long-term preservation. The natural diversity is critical because of the habitat destruction, population growth and unplanned industrialization. Despite its economical, ecological and medicinal values little is known about the genetic structure of *Piper* species, thus it is of utmost importance to characterize the genetic variation of different genotypes for better conservation strategy. The present investigation was carried out to study the genetic and phytochemical characterization of eight different genotypes of *Piper* species/accessions collected from the Eastern part of India (Odisha).

Materials and methods

Plant materials: Eight different genotypes/accession of *Piper i.e. P. chaba* (P1), *P.* spp. Accession -1 (P2), *P.* spp. Accession -2 (P3), *P. longum* (P4), *P. nigrum* (P5) *P. betle* var. Utkal Sudhama (P6), *P. betle* var. Astarangi Balunga (P7), *P. betle* var. Godi Balunga (P8) were collected from different regions of Eastern part of India for genetic analysis and phytochemical experiment. The voucher specimen is also given as supporting documents and identified by Dr. G. Das, Officer-in-Charge, AICRP in Betlevine, OUAT, Bhubaneswar. The morphological characteristics of different *Piper* genotypes/accessions are given in Table 1. Young leaves were collected and kept in the plastic bags in deep freezer (-20 °C) for genomic DNA extraction. Ten grams of leaves and fruits of each genotypes were also collected for photochemical analysis.

Isolation of genomic DNA and PCR amplification: Genomic DNA was extracted from young leaves using the *N*-cetyl-*N*-*N'N*-trimethyl ammonium bromide (CTAB) method described with modifications (Doyle and Doyle, 1990). Two grams of fresh leaf material were washed in distilled water and subsequently rinsed with 80% (v/v) ethanol and then ground in liquid nitrogen. Ten mL of preheated extraction buffer [4% (w/v) CTAB, 0.2 % β-mercaptoethanol (v/v), 100 mM Tris-HCl pH 8.0, 2 mM EDTA, 1.4 M NaCl] were then added per 2 g of leaf powder material and incubated for two hours at 65°C. The DNA pellet was resuspended in 200-300 µL of Tris-EDTA buffer. DNA quantification was performed by visualization under UV light, after electrophoresis on 0.8% (w/v) agarose gel. The resuspended DNA was then diluted in sterile distilled water to 5 ng/µL concentration for use in amplification reactions. Twenty-five synthesized ISSR primer (M/S Emerck Bioscience, India) were initially screened using one genotype *P. nigrum* to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the species/accessions. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

A PCR with a single primer was carried out in a final volume of 25µL containing 20 ng template DNA,100 µM of each deoxyribonucleotide triphosphate, 20 ng of primer, 1.5 mM $MgCl_2$, 1×Taq buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.001% gelatin), and 0.5U Taq DNA polymerase (M/S Emerck Bioscience, India). Amplification was performed in a PTC-100 thermal cycler (Peqlab, Germany) programmed for a preliminary 2 min denaturation step at 94 °C, followed by 40 cycles of denaturation at 94 °C for 20 sec, annealing at the required temperature for 30 sec depending on the primer and extension at 72 °C for 1 min, and finally amplification at 72 °C for 10 min. The detailed primer sequences are presented in Table 2. Amplification products were separated alongside a molecular

Table 1. Morphological characteristics of different *Piper* species / accessions collected from Eastern Part of India

Name of species/ accessions	Shape of leaf	Size L × B (cms)	No. of main vein	Length of petiole (cm) (Mean ± SE)*	Marginal lamina	Tip apex	Colour of leaf	Leaf texture	Internodes length (cm) Mean±SE)*	Branching habit
P. chaba	Lanceolate	11.5 × 5	9.0	1.52±0.6*	Entire	Acute	Green, petiole pink in colour	Smooth	3.24±0.8*	Profuse
P. spp. Accession-1	Cordate	10.23 × 10.36	7.0	5.33±0.7	Entire	Acute	Dark green	Smooth	9.52±0.4	Profuse
P. longum	Ovate	6.9 × 6.8	7.0	3.26±0.4	Entire	Acute	Dark green	Smooth	5.73±0.7	Profuse
P. spp. Accession-2	Cordate	11.66 × 7.93	7.0	5.51±0.6	Entire	Pointed	Light green	Rough	7.63±0.6	Sparse
P. nigrum	Ovate	10 × 6	7.0	6.50±0.3	Entire	Acute	Dark green	Smooth	5.74 ±0.5	Profuse
P. betle var. Utkal Sudhama	Cordate	16.75 × 14.1	7.0	10.75±0.5	Entire	Acute	Deep Green	Smooth	9.21±0.5	Sparse
P. betle var. Astarangi Balunga	Cordate	16.4 × 15	7.0	7.34±0.7	Entire	Acute	Green	Smooth	7.1±0.6	Sparse
P. betle var. Godi Balunga	Ovate	18.4 × 14.9	7.0	11.2±0.3`	Entire	Acute	Green	Smooth	8.0±0.8	Sparse

Table 2. Total number of amplified fragments and number of polymorphic fragments generated by PCR using ISSR primers

ISSR Primer	Primer sequence	Total number of bands	Number of polymorphic bands	Polymorphism percentage	Number of unique bands	Band range |(bp)
USB-807	$(AG)_8T$	10	07	70.0	01	400-1000
USB-808	$(AG)_8C$	09	06	66.6	01	600-2000
USB-810	$(GA)_8T$	08	07	87.5	01	500-1200
USB-811	$(GA)_8C$	09	08	88.9	02	500-1200
USB-815	$(CT)_8G$	10	09	90.0	02	400-2000
USB-835	$(AG)_8CT$	08	03	37.5	01	500-1500
USB-836	$(AG)_8TC$	10	09	90.0	02	400-1500
USB-837	$(AG)_8GC$	09	07	77.7	01	400-1000
USB-838	$(GA)_8TTC$	12	11	91.6	01	300-1500
USB-839	$(GA)_{12}C$	09	08	88.9	02	600-1500
USB-840	$(CT)_8T$	08	07	87.5	-	700-1500
USB-841	$(AG)_8GCG$	10	09	90.0	-	500-2000
USB-842	$(AG)_8TA$	07	06	85.7	01	800-2000
USB-843	$(GA)_8AT$	09	08	88.9	02	500-1200
USB-844	$((GA)_8CG$	10	09	90.0	03	400-1500
		138	114	82.6	20	700-2000

1- *P. chaba*, 2- *P.* spp var. Accession-1, 3- *P.* spp var. Accession-2, 4- *P. longum*, 5- *P. nigrum*, 6- *P. betle* var. Utkal Sudhama, 7- *P. betle* var. Astarangi Balunga, 8- *P. betle* var. Godi Balunga.

weight marker (1.0 kb plus ladder, M/S Emerck Bioscience, India) by 1.2% (w/v) agarose gel electrophoresis in 1x TAE (Tris Acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through the Gel Doc System (UVTECH, UK) and the amplification product sizes were evaluated using the software Quantity one. Data were recorded as presence (1) or absence (0) of DNA fragment products from the photographic examination. Each amplification fragment was named by the source of the primer, the primer number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. A pair-wise matrix of distance between landraces was determined for the ISSR data using Dice Coefficient of similarity. The similarity measures were subjected to unweighted pair group method for arithmetic averages (UPGMA) and plotted in a phenogram using NTSYS-pc Ver. 2.1 (Rolhf, 2001).

Isolation of secondary metabolites: One g of leaf and fruit samples were air dried and were macerated in mortal and pestle to obtain a fine powder. The powdered plant material was loaded in the soxlet apparatus and then fitted into a round flask containing methanol. The solvent was boiled gently (60-80 ^{0}C) over a heating mantle using adjustable rheostat. The extraction was continued until complete extraction was done within 8 to 10 hrs. After extraction, extract was collected and methanol was allowed to evaporate. Dry mass of extract was weighed and stock was made of 1 mg/mL concentration. This was used for HPTLC analysis for presence of standard piperine and piperidine. The standard was purchased from M/S Sigma, USA (99% purity).

Stock solution (1 mg/mL) of standard compound piperine was prepared individually in methanol and different concentration was spotted onto TLC plates in order to prepare the calibration graph. Aluminium plate pre-coated with silica gel 60 F254 TLC (10 x10 cm) (EMerck, India) was used as a stationary phase. A Linomat IV (Camag, Muttenz, Switzerland) automatic TLC applicator was used to apply samples and standards onto the TLC plates under a flow of nitrogen gas. The application parameter was identical for all analysis performed and the delivery speed of the syringe was 10s/µL. The extract and standard solution were applied 1.5 cm away from lower edge of the plate with the help of micro capillary tube. The solvent system used to identify piperine was Toluene: Ethyl acetate (7:3 v/v) under laboratory condition. The loaded plates were then placed vertically in the chamber previously saturated with solvent system for 30 min. After the solvent moved about 90% of length, the plates were taken out and dried at room temperature.

Developed plates were dried in air stream and immersed in 5% concentrated ethanolic sulphuric acid. After drying, the plates were heated at 110 ^{o}C for 15-20 min to develop the colour spots. For quantitative determination, spots corresponding to standards were scanned using a Camag TLC Scanner 3 at 254 nm and 343 nm wavelength with a slit size of 6 x 0.4 mm.

The R_f values were calculated using formula

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Results and discussion

The present investigation was to optimize the primer screening for evaluation of genetic relationship between eight *Piper* species / accessions. The results imply that the extraction and purification of genomic DNA were difficult due to presence of secondary metabolites and essential oil content. A modified CTAB method with higher concentration of CTAB (4%), EDTA (50 mM) and 1% 2-mercaptoethanol was used for extraction of genomic DNA. Significant quantities of DNA were successfully extracted by this modified method that varied from 200 to 800 ng in different *Piper*

species. The reproducibility of ISSR amplification was detected by performing separate runs of PCR with DNA extraction from different preparation. No significant differences were observed in different experiments although occasional variation in the intensities of individual DNA fragments occured. Bands with same mobility were considered as identical fragments receiving equal values regardless of their staining ability. Fifteen ISSR primers were selected as they generated 138 clear and scorable bands with an average of ~ 9 bands per primer. Out of which, 114 bands were polymorphic revealing 82.6 % polymorphism. The banding pattern by ISSR primers *i.e.* USB-839, USB-836, USB-838, USB-810, USB-841 and USB-835 are presented in Fig. 1. The result shows that ISSR marker was efficient enough to distinguish eight species/accessions of *Piper* and in revealing molecular relationship among themselves. The similarity matrix of ISSR data after multivariate analysis using Nei and Li's coefficient is presented in Table 3. The similarity value ranged from 0.41 to 0.98. The similarity matrix obtained in the present study was used to construct a dendrogram with the UPGMA method (Fig. 2). The dendrogram generated by ISSR was broadly divided into two major groups with 47% similarity. *Piper* species Accession-1, morphologically and genetically distinct from the rest of 7 species/accessions, grouped into single group. At the molecular level, *Piper* spp. Accession-1 is having unique ISSR bands. The remaining seven species/accessions positioned in group-II were again divided into two clades. The first clad having six species/accessions (*P. chaba*)*, P. longum, Piper* spp Accession-1*, P. betle* var. Utkal Sudhama*, P. betle* var. Astarangi Balunga and *P. betle* var. Godi Balunga and other clad having one species *P. nigrum*. *P. betle* var. Godi Balunga and *P. betle* var. Astarangi Balunga were grouped together with 100 % similarity

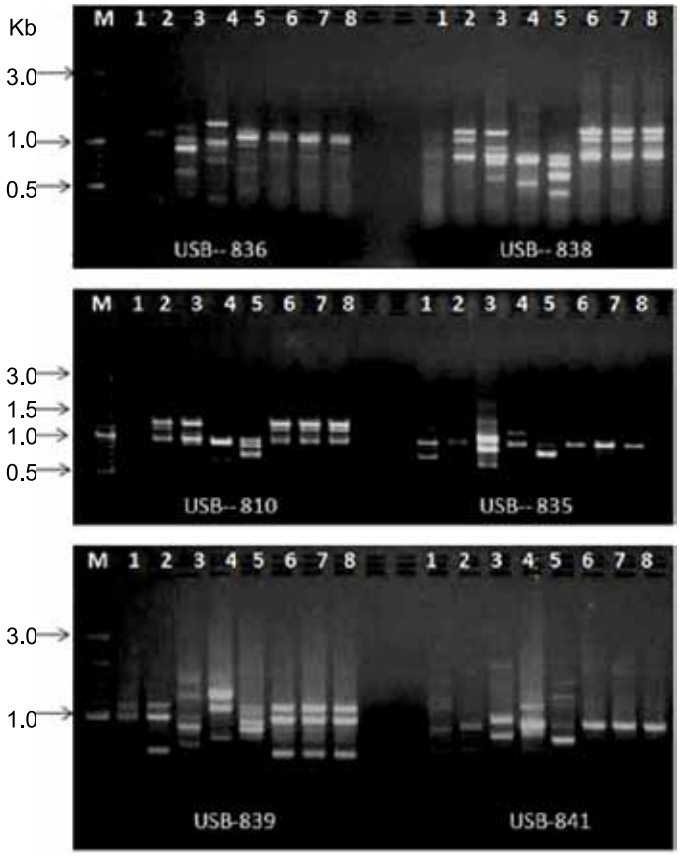

Fig.1. ISSR banding pattern in 8 species/accessions of *Piper* obtained from PCR amplification.

Table 3. Similarity matrix of 8 species/accessions of *Piper* generated by ISSR markers

	P1	P2	P3	P4	P5	P6	P7
P2	0.71						
P3	0.48	0.50					
P4	0.66	0.60	0.41				
P5	0.55	0.60	0.45	0.48			
P6	0.64	0.90	0.50	0.57	0.60		
P7	0.62	0.88	0.48	0.55	0.59	0.98	
P8	0.62	0.88	0.48	0.55	0.59	0.98	1.00

P1- *P. chaba*, P2- *P.* spp Var. Accession-1, P3- *Piper* spp var. Accession-2, P4- *P. longum*, P5- *P. nigrum*, P6- *P. betle* var. Utkal Sudhama, P7- *P. betle* var. Astarangi Balunga, P8- *P. betle* var. Godi Balunga.

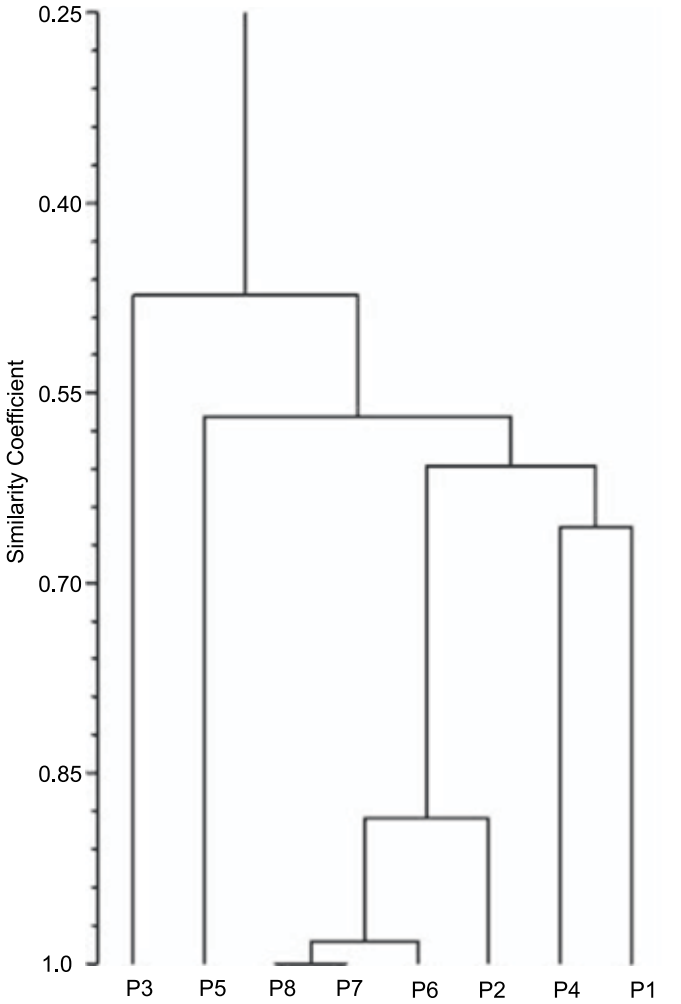

Fig.2. Dendrogram showing the cluster analysis of eight species/accessions of *Piper* using ISSR markers. P1- *P. chaba*, P2- *Piper* spp var. Accession-1, P3- *Piper* spp Var. Accession-2, P4- *P. longum*, P5- *P. nigrum*, P6- *P. betle* var. Utkal Sudhama, P7- *P. betle* var. Astarangi Balunga, P8- *P. betle* var. Godi Balumga.

at genetic level, whereas, *P. betle* var. Utkal Sudhama had 97% similarity with P7 and P8. Morphologically, they are very close with each other. The genotype like *Piper* spp Accession -1 formed single cluster with 88% similarity with *P. betle* var. Astarangi Balunga and Var. Godi Balunga. The dendogram shows that there was distant variation within the species of *P. chaba* and *P. longum* and also 65% similarity genetically with *Piper* spp Accession-1, *P. betle* vars. Utkal Sudhama*,* Astarangi Balunga and Godi Balunga.

The piperine (P1 and P2) content was examined in different species of *Piper* through TLC and HPTLC analysis. On the basis of phytochemical analysis, the piperine (P1 & P2) content

varied from species to species and also differs in the source of explant. Two peaks were observed during the chromatography analysis *i.e.* piperine -1 & piperine-2 as reported earlier (Stohr *et al*., 2001; Suthar *et al*., 2003). The piperine-1 content was maximum in *P. chaba* fruit as compared with other species. However, the piperine-2 content was more in *P. nigrum* fruit (Table 4). On the basis of TLC and HPTLC analysis, it was observed that piperine-2 content varied in leaf and fruit extract of different species of *Piper* as compared to standard (Fig. 3).

The R_f value and unit area varied in different species/accession. The maximum piperine content was observed in *P. chaba* and *P. longum*. The range of phytochemical variation in plant species help in the selection of elite clone.

The present investigation was to identify the elite clones of different species/accession of *Piper* through molecular and phytochemical analysis. Among the all tested primer, fifteen ISSR primers were selected for good amplification and generated 138 clear and scorable bands with 82.6 % polymorphism. The

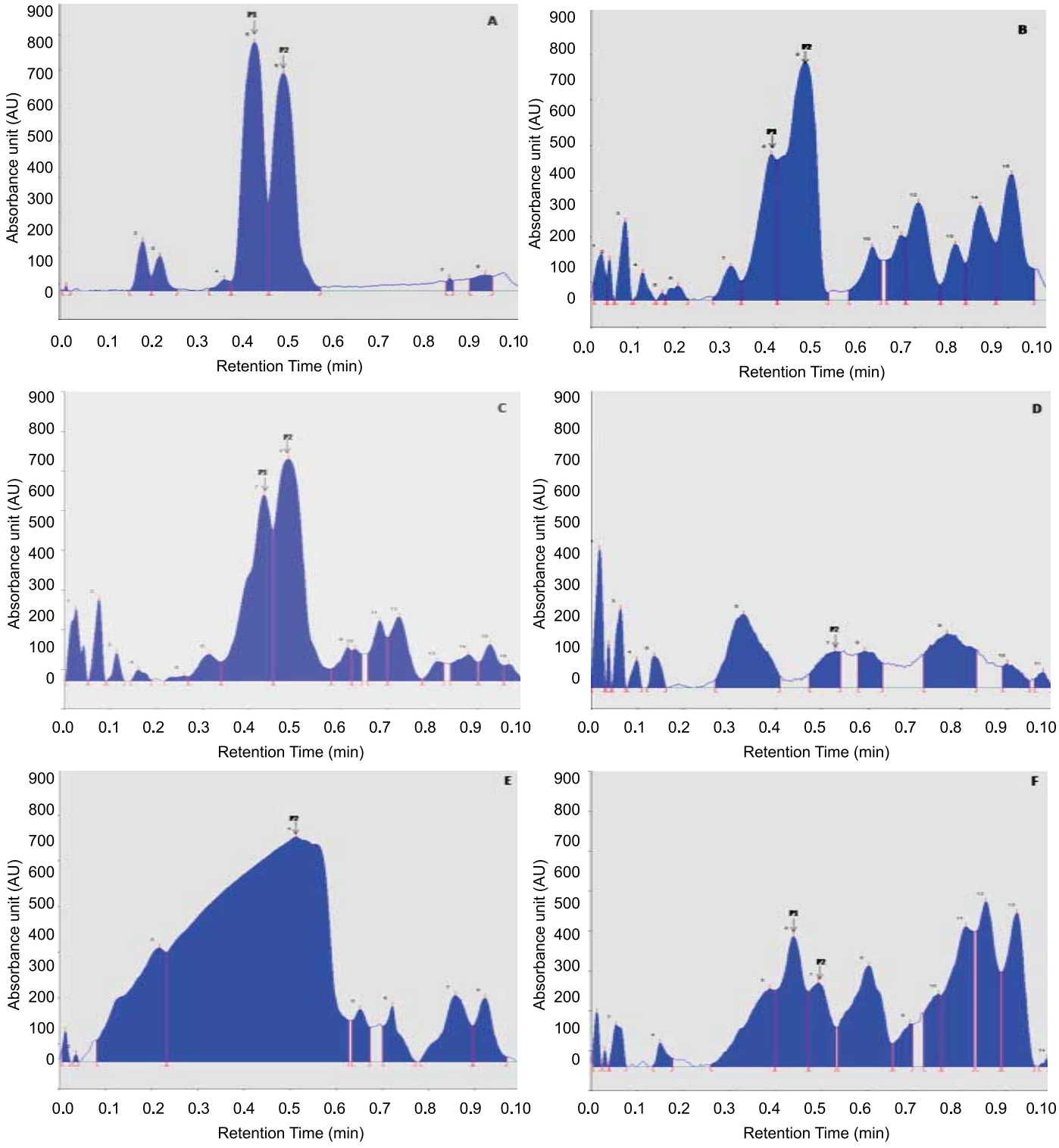

Fig. 3 A-F**:** HPTLC analysis of piperine content (P-1 & P-2) in different *Piper* species. A- Standard piperine (P-1 & P-2), *P. longum* leaf (B), Fruit (C), *P. nigrum* leaf (D), fruit (E), *P. chaba* leaf (F)

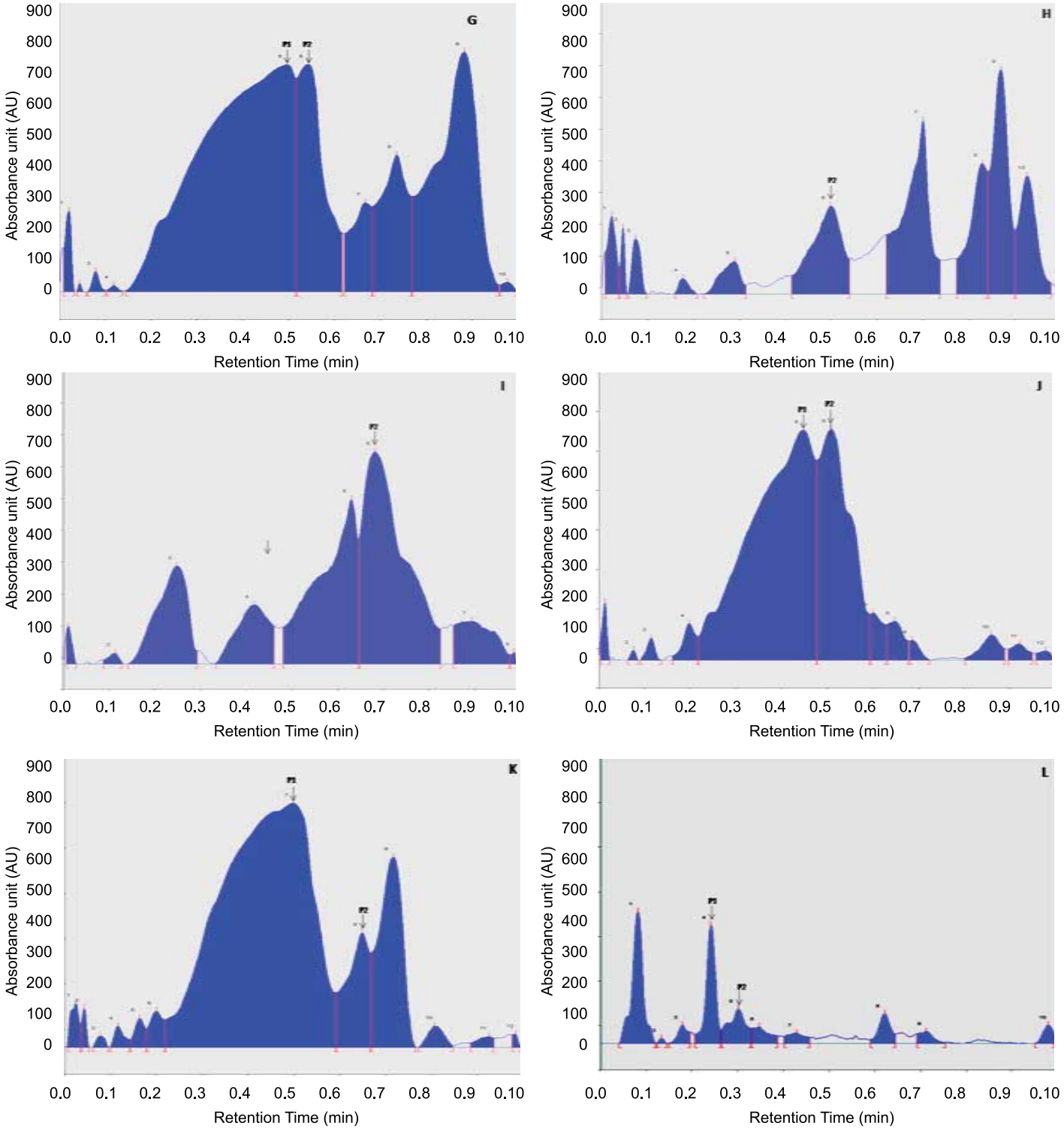

Fig. 3. G–L: HPTLC analysis of piperine content (P-1 & P-2) in different *Piper* species. *P. chaba* Fruit (G), *P. betle* var. Utkal Sudhama (Leaf) (H), *P. betle* var. Utkal Sudhama Fruit (I), *P. betle* var. Astarangi Balunga (Leaf) (J), Fruit (K) and *P. betle* var. Godi Balunga (leaf) (L) compare with standard (A)

genetic variation through ISSR markers has been highlighted in a number of medicinal plants (Bai *et al*., 1997; Pal and Raychaudhuri, 2003; Rout, 2006).

The variation in genetic differences estimated by ISSR markers in this study are close in resemblance with the result of previous study in *Piper* species and other medicinal plants (Chawdhury *et al*., 2014; Jiang and Liu, 2011; Rahman *et al*., 2012). The dendrogram shows that there was distant variation within the species of *P. chaba* and *P. longum* with 65% similarity with *Piper* spp Accession-1, *P. betle* vars. Utkal Sudhama, Astarangi Balunga and Godi Balunga. On the basis of phytochemical analysis, the piperine (P1 & P2) content varied from species to species and also differ in the source of explant. Two peaks were observed during the chromatography analysis *i.e.,* piperine -1 and piperine-2 as reported earlier (Hamrapurkar *et al*., 2011; Rashmi *et al*., 2010). The piperine-1 content was maximum in *P. chaba* fruit as compared with other species. However, the piperine-2 content was more in *P. nigrum* fruit. The maximum piperine content was observed in *P. chaba* and *P. longum*. Pino *et al*. (2003) observed

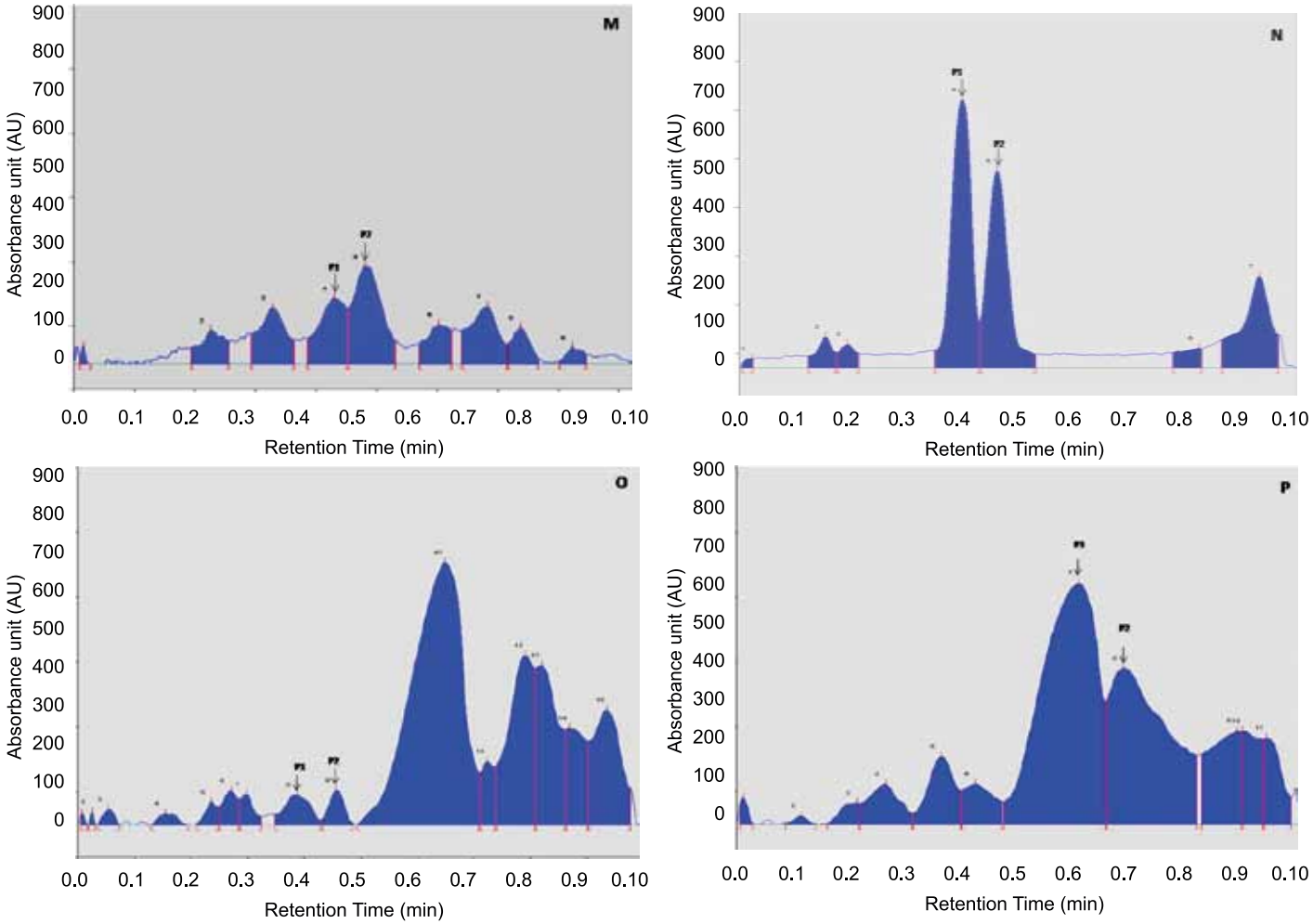

Fig. 3. M-Q: *P. betle* var. Godi Balunga (Fruit) (M), *Piper s*pp Accssion-1 (leaf) (N), fruit (O), *Piper s*pp Accssion-2 (Leaf) (P) , *Piper s*pp Accssion-2 (Fruit) (Q) compare with standard (A)

that the major components of the essential oil obtained from the aerial parts of *P. nigrum* were gluulol, a-pinene, ß-caryophyllene and a-terpinene. Piperine was the first amide to be isolated from *Piper* species. Piperine, the major active principle of black pepper, is closely related in structure to the known natural carcinogens-safrole, estragole and methylengenol which are also widely distributed in spices and plant oils. The phytoconstitutes like piperine content was varied differently in fruits of *P. nigrum* and *P. longum* as reported earlier (Khare *et al*., 2006). The range of phytochemical variation in plant species help in the selection of elite clone. However, ISSR markers provide a reliable method for identification of species than morphological characters. This investigation as an understanding of the level and partitioning of genetic variation within the species / accessions would provide an important input into determining efficient management strategies. The genetic variability in a gene pool is normally considered as being the major resource available to breeders for *Piper* improvement program.

Acknowledgements

The authors acknowledge Department of Biotechnology, Ministry of Science & Technology, Govt. of India for PG-HRD teaching grant. The authors are also thankful to Officer-in-Charge, Central Laboratory Facility at OUAT for HPTLC analysis.

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Table 4. Comparison of piperine content in leaf and fruit of *P. longum, Piper* sps. Accssion-1, *Piper* sps. Accssion-2, *P. nigrum, P. chaba* and *P. betle* Vars. Utkal Sudhama, Astarangi Balunga, Godi Balunga. The data represent the mean value of three independent experiment. (* - indicate no content)

Sample (mg/mL) (5µL used in each case)	Piperine 1			Piperine 2		
	Rf	Area unit	Content (%) (Mean ± SE)	Rf	Area unit	Content (%) (Mean ± SE)
Standard piperine	0.42 (0.37-0.45)	27923.9	1.0	0.48 (0.45-0.56)	26133.00	1.0
P. longum (leaf)	0.39 (0.33-0.40)	11558.8	0.41±0.3	0.46 (0.40-0.51)	30056.40	1.15±0.12
P. longum (fruit)	0.43 (0.34-0.45)	22651	0.82±0.4	0.49 (0.45-0.58)	30052.30	1.14±0.34
P. nigrum (leaf)	*	*	*	0.53 (0.47-0.54)	2504.80	0.05±0.05
P. nigrum (fruit)	*	*	*	0.51 (0.23-0.63)	116432.80	4.45±0.54
P. chaba (leaf)	0.44 (0.40-0.47)	7201.00	0.25±0.08	049 (0.47-53)	4126.7	0.15±0.19
P. chaba (fruit)	0.50 (0.14-0.52)	106854.0	3.82±0.64	0.54 (0.52-0.62)	30262.4	1.15±0.56
P. betle var. Utkal Sudhama (leaf)	*	*	*	0.50 (0.41-0.54)	8582.50	0.32±0.12
P. betle var. Utkal Sudhama (fruit)	*	*	*	0.50 (0.39-0.51)	12551.6	0.25±0.06
P. betle var. Astarangi Balunga (leaf)	0.11 (0.14-0.24)	18472.8	0.21±0.12	0.36 (0.24-0.38)	10,256.6	0.35±0.13
P. betle var. Astarangi Balunga (fruit)	0.14 (0.18-0.30)	23045.4	0.31±0.04	0.39 (0.30-0.41)	8166.40	0.38±0.10
P. betle var. Godi Balunga (leaf)	0.13 (0.28-0.32)	3532.6	0.35±0.10	0.42 (0.32-0.40)	2166.8	0.43±0.7
P. betle var. Godi Balunga (fruit)	0.17 (0.25 -0.35)	3718.7	0.37±0.08	0.45 (0.35-0.44)	5152.3	0.48±0.32
Piper sps. Accssion-1 (leaf)	0.41 (0.36-0.48)	6550.4	0.56±0.18	0.46 (0.48-0.55)	7232.5	0.36±0.22
Piper sps. Accssion-1 (fruit)	0.36 (0.32-0.40)	3651.2	0.40±0.14	0.51 (0.40-0.52)	4052.10	0.42±0.18
Piper sps. Accession-2 (leaf)	0.38 (0.32-0.41)	15554.5	0.44±0.14	0.55 (0.41-0.58)	18953.1	0.82±0.21
Piper sps. Accession-2 (fruit)	0.35 (0.38-0.48)	4974.6	0.50±0.11	0.44 (0.48-0.58)	4536.4	0.45±0.11

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Received: May, 2016; Revised: July, 2016; Accepted: August, 2016