days after the full moon day, the moon must have risen 156 min (52 × 3) later than on full moon night. So the moonrise must be approximately at 9 p.m. At that time the rising zodiacal constellation was Virgo. But the birth time of Shivaji Maharaj was in the evening, after sunset. So at that time the earlier constellation Leo must be rising on the eastern horizon. In the history of Shivaji Maharaj, his birthday is mentioned as 19 February 1630. However, the above references do not match with the star patterns on this date as extrapolated back in the Gregorian calendar. They match very precisely on 1 March 1630 (ten days later).

Another and probably more accurate example is the partial solar eclipse visible from fort Raigad in the year 1680. In the various biographies of Shivaji Maharaj, it is mentioned that partial solar eclipse was visible on 20 March 1680. From the Gregorian calendar there were no chances of an eclipse on that day because the sun was in Pisces and moon was in Libra. But on 30 March 1680, an eclipse did occur. It was in the constellation of Pisces. From Raigad the eclipse started at 4 h 37 min in the evening. The sun was 30 degrees above the horizon. The mid-eclipse time was 5 h 41 min in the evening. The sun was only 15 degrees above the horizon. The eclipse ended at time 6 h 38 m in the evening when the sun was only 1.5 degrees above horizon[1]. This observation exactly matches with the documented history.

Table 1 gives many other events in the life of Shivaji Maharaj, which confirm our observations. All the *tithis* are taken from ref. 4.

We therefore conclude that the manner in which dates are calculated in the historical timescales has carried the error of Julian and Gregorian calendar dates. Hence care must be taken while calculating the English calendar dates of the past events from periods earlier than late eighteen century.

1. Oppelzer, T. R. V., *Canon of Eclipses*, 1887.
2. Abhyankar, K. D., *Sci. Rep.*, 1976, **13**, 424.
3. Shylaja, B. S., *Bull. Astron. Soc. India*, 1997, **25**, 601.
4. Purandare Balasaheb, *Raja Shivachhatrapati*, Purandare Prakashan, Pune, 1993.
5. Mahendale, G. B., *Shree Raja Shivachhatrapati*, 1930, vol. 1.

ACKNOWLEDGEMENT. We are grateful to the referee for valuable comments.

Received 29 July 2002; revised accepted 4 December 2002

MOHAN APTE[†]
PARAG MAHAJANI[#]
M. N. VAHIA[‡,*]

[†]*Rupali Society,*
Vile Parle (East),
Mumbai 400 057, India
[#]*Jeevan Shobha,*
Jain Mandir Cross Road,
Borivili (East),
Mumbai 400 066, India
[‡]*Department of Astronomy and Astrophysics,*
Tata Institute of Fundamental Research,
Homi Bhabha Road,
Colaba, Mumbai 400 005, India
**For correspondence.*
e-mail: vahia@tifr.res.in

Molecular identification of *Phytophthora nicotianae* isolates causing leaf rot of betelvine (*Piper betle* L.)

Betelvine (*Piper betle* L.) is an important cash crop in Asia, with trade worth Rs 700 crores in India alone[1]. One of the major limiting factors in yield is foot and leaf rot disease caused by *Phytophthora parasitica* var. *piperina*[2] that sometimes causes 100% losses. The importance of molecular diagnostics in the management of this crop has been emphasized by Johri *et al*.[3] because morphological identification is laborious and requires high level of expertise and could also lead to false determination. In this communication, we attempted a survey of betelvine conservatories for natural occurrence of the disease, isolation of pathogen, genomic DNA extraction, polymerase chain reaction (PCR), Southern hybridization and restriction fragment length polymorphism (RFLP) analysis of the PCR products to establish the identity at molecular level and the relationship/differentiation between Indian and other known isolates of *Phytophthora*.

The pathogen was isolated on PDA plates at 25 ± 2°C from naturally infected betelvine leaves showing typical concentric rings, and pathogenicity was established on *P. betel*. Genomic DNA of fungal isolates was extracted as described earlier by Kistler *et al*.[4], with slight modification. The extracted DNA was treated with RNAase (10 mg/ml), Proteinase K (10 mg/ml), reprecipitated and finally suspended in 20 µl TE. DNA obtained was quantified by taking OD at 260 nm using a spectrophotometer.

PCR reactions were performed using ITS4 and ITS6 universal primers described earlier[5]. ITS6 was a universal primer designed to improve the amplification of a part of rDNA of Oomycota; it amplifies a ~ 900 bp product in combination with ITS4 primers. PCR reaction mixture (50 µl) contained template (50–70 ng), Taq DNA polymerase (3 u/µl), buffer (10 X), dNTP (10 mM), $MgCl_2$ (25 mM), primer (25 pmol/µl each). The initial denaturation was done at 94°C for 3 min followed by 25 cycles with the conditions of 94°C for 1 min (denaturation), 52°C for 1 min (annealing), 72°C for 1.30 min (extension) and a final extension at 74°C for 5 min. PCR products obtained were analysed by electrophoresis on the 1.0% agarose gel. For Southern hybridization, DNA bands obtained on agarose gel were transferred to Hy-bond membrane (Amersham-Pharmacia, UK) and hybridized with radiolabelled probe, prepared by random primer extension method as described earlier[6], using genomic DNA of a known *P. infestans* obtained from SCRI, Dundee, UK. For RFLP analysis, the PCR amplicons obtained from DNA of the fungal isolates and DNA of *P. infestans* (Scottish isolate) were run on LMP agarose gel, eluted and digested by *Alu*I, *Msp*I, *Taq*I restriction enzymes separately as suggested[7,8]. The digested DNA samples were analysed by electrophoresis on 1.3% agarose gel.

During our field survey (2001–2002), natural infection of leaf rot disease of betelvine exhibiting typical concentric rings (Figure 1) was observed at Lucknow and Mahoba research stations in Uttar Pradesh. The intensity of the disease was about 40–50%. Pathogenicity of the pathogen isolated was successfully established as similar leaf rot symptoms appeared on inoculated leaves of betelvine after five days of incubation.

Approximately 100 ng/µl of genomic DNA from fungal isolates was extracted by following the protocol of Kistler *et al.*[4] PCR reactions using universal primers revealed an amplicon of about 900 bp in DNA samples of both the fungal isolates (Figure 2). Since the primers were universal to amplify an ITS region of rDNA of Peronosporales (i.e. *Pythium*, *Phytophthora*, etc.) and the PCR amplicons obtained from both the fungal isolates were of expected size (~ 900 bp) with the ITS4 and ITS6 primers as mentioned by Cooke *et al.*[9], the fungal isolates may be considered as Peronosporales. Southern hybridization tests of PCR products revealed strong signals with the probe prepared from genomic DNA of *P. infestans* (figure not included); the fungal isolates may be placed under the *Phytophthora* genus.

Figure 1. Infected betelvine leaves showing typical concentric rings and rotting symptoms.

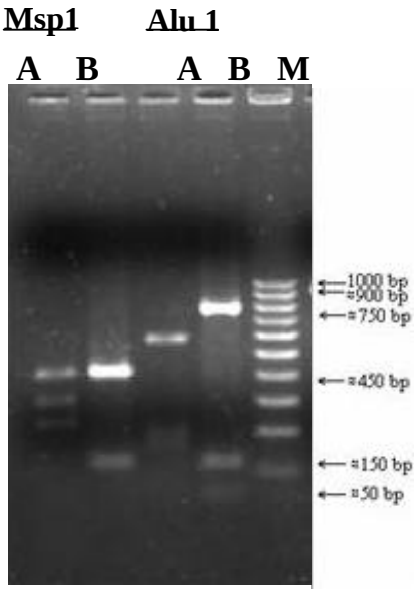

Figure 3. RFLP profile of *Phytophthora* isolates from SCRI and NBRI A, *Phytophthora* isolate 10 from SCRI; B, *Phytophthora* isolate 1 from NBRI; M, 100 bp ladder.

The results of RFLP analysis of the PCR products of isolate 1 and SCRI isolate are shown in Figure 3. Digestions with *Alu*I, *Msp*I and *Taq*I revealed a clear-cut difference in their restriction patterns. *Alu*I yielded three bands of approximately ~ 750, 150 and 50 bp size in *Phytophthora* isolates under study, whereas three bands of ~ 540, 200 and 160 bp were observed in *P. infestans* (Scottish isolate). *Msp*I digestions revealed three bands of ~ 410, 390 (seen as one band of about 400 bp) and 120 bp in *Phytophthora* isolates under study, whereas there were three clear bands of ~ 400, 300 and 200 bp in *P. infestans* (Scottish isolate). *Taq*I yielded four bands of ~ 300, 200, 150 and 100 bp in *Phytophthora* isolates under study, whereas five clear bands of ~ 300, 280, 150, 100 and 60 bp *P. infestans* (Scottish isolate) were observed. The 60 bp band could not be seen, may be due to poor resolution (figure not included). Results of isolate 4 were identical to isolate 1 (Table 1). Comparative data of RFLP analysis of isolates 1 and 4 with *P. infestans* (SCRI isolate) and *P. nicotianae* published earlier by Cooke *et al.*[8] are summarized in Table 1. It indicates a similar RFLP pattern for both the Indian isolates with *P. nicotianae*, but not with *P. infestans*. The restriction pattern of both the Indian isolates under study was also compared with other species of *Phytophthora* (not included in Table 1). The present studies of RFLP suggest our isolate as *P. nicotianae*. Comparison of DNA digest pattern with the known profile of *Phyto-*

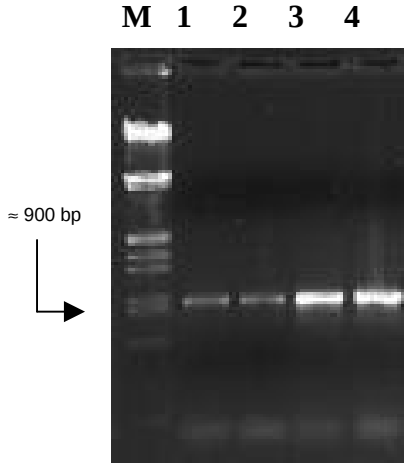

Figure 2. PCR amplication of *Phytophthora* isolates. M, Lambda DNA *Eco*RI/ *Hin*dIII double digest; Lanes 1, 2, SCRI isolates 10; Lanes 3, 4; NBRI isolates 1 and 4.

Table 1. Comparative RFLP analysis of PCR products obtained with data published recently* by Cooke *et al.*[8]

Species \ Enzyme	*Alu*1	*Msp*1	*Taq*1
P. infestans (SCRI isolate)	~ 540 bp, 544 bp*, ~ 200 bp, 194 bp*, ~ 160 bp, 160 bp*	~ 400 bp, 396 bp*, ~ 300 bp, 288 bp*, ~ 200 bp, 221 bp*	~ 300 bp, 309 bp*, ~ 280 bp, 283 bp*, ~ 150 bp, 155 bp*, ~ 100 bp, 99 bp*, ~ 60 bp, 59 bp
P. nicotianae (isolate 1)	~ 750 bp, 745 bp*, ~ 150 bp, 117 bp*, ~ 50 bp, 52 bp*	~ 410 bp, 404 bp*, ~ 390 bp, 390 bp*, ~ 120 bp, 120 bp*	~ 300 bp, 317 bp*, ~ 200 bp, 193 bp*, ~ 150 bp 148 bp*, ~ 100 bp, 100 bp*, 59 bp*
P. nicotianae (isolate 4)	~ 800 bp, 745 bp*, ~ 150 bp, 117 bp*, ~ 50 bp, 52 bp*	~ 410 bp, 404 bp*, ~ 390 bp, 390 bp*, ~ 120 bp, 120 bp*	~ 300 bp, 317 bp*, ~ 200 bp, 193 bp*, ~ 150 bp, 148 bp*, ~ 100 bp, 100 bp*, 59 bp*

phthora spp. is helpful for identification of Indian isolates of *P. nicotianae* based on similarities with *P. nicotianae*[8] and disimilarities with SCRI isolates published earlier as *P. infestans***.**

Furthermore, both the isolates were identified morphologically at the Indian Agricultural Research Institute, New Delhi as *P. parasitica* (Accession number: ITCC-5232 and ITCC-5233; the name of *P. parasitica* has been corrected as *P. nicotianae* Breda de Haan), which supported our molecular identification studies.

Bonants *et al.*[10] suggested that detection of fungal pathogens by PCR is at least 10-fold more sensitive than ELISA. Moreover, serological techniques developed to detect the *Phytophthora* are generally genus-specific[11]. Molecular identification studies of the pathogen causing leaf rot of betelvine will be helpful to develop PCR-based diagnostics for sensitive, rapid and reliable detection of *Phytophthora* spp. directly from *P. betle* leaves as well as soil samples which may contribute an important role for implementation of the efficient control strategies.

1. Balasubrahmanyam, V. R., Johri, J. K., Rawat, A. K. S., Tripathi, R. D. and Chaurasia, R. S., Betelvine (*Piper betle* L.) National Botanical Research Institute, Lucknow, 1994.
2. Saksena, S. B., *Indian Phytopathol.*, 1977, **30**, 1–16.
3. Johri, J. K., Singh, H. B. and Srivastava, M., *Advances in Plant Disease Management* (eds Udit Narayan, Kumud Kumar and Mukesh Srivastava), Advance Publishing Concepts, New Delhi, 2000, pp. 175–183.
4. Kistler, H. C., Momol, E. A. and Benny, U. A., *Phytopathology*, 1991, **81**, 331–336.
5. White, T. J., Bruns, T., Lee, S. and Taylor, J., in *PCR Protocols: A Guide to Methods and Applications* (eds Innis, M. A. *et al.*), Academic Press, New York, 1990, pp. 315–322.
6. Feinberg, A. P. and Vogelstein, B., *Anal. Biochem.*, 1983, **132**, 6–12.
7. Cooke, D. E. L. and Duncan, J. M., *Mycol. Res.*, 1997, **101**, 667–677.
8. Cooke, D. E. L., Duncan, J. M., Williams, N. A., Hagenaar-de Weerdt, A. and Bonants, P. J. M., OEPP/EPPO Bulletin, 2000, vol. 30, pp. 519–523.
9. Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G. and Brasier, C. M., *Fungal Genet. Biol.*, 2000, **30**, 17–32.
10. Bonants, P., deWeerdt, M. H., VanGent-Pelzer, M., Lacourt, I., Cooke, D. and Duncan, J., *Eur. J. Plant Pathol.*, 1997, **103**, 345–355.
11. Grote, D. and Gabler, J., *J. Plant Dis. Protect.*, 1999, **106**, 445–454.

ACKNOWLEDGEMENTS. We thank Shaukat Hussain and J. Duncan, SCRI, Dundee, UK for providing DNA of *Phytophthora infestans* and ITS4 and ITS6 primers as a gift to S.K.R., and also for valuable suggestions. We also thank the Director, National Botanical Research Institute, Lucknow for his keen interest and for providing facilities.

Received 28 May 2002; revised accepted 26 November 2002

A. TRIPATHI
R. SINGH*
S. K. RAJ*
A. P. SINGH
J. K. JOHRI†

Plant Protection and Quarantine, and
**Molecular Virology Division,*
National Botanical Research Institute,
Lucknow 226 001, India
†*For correspondence*
e-mail: jjohri@rediffmail.com.

Mist net captures of the rarest fruit bat *Latidens salimalii*

Next to rodents, bats are the most numerous mammals in the world. Koopman[1] recognized a total of 888 species of bats. However, numbers from 800 to 1000 are frequently quoted for the bat species[2–4]. According to Mickleburgh *et al.*[5], there are 1001 species of bats in the world. India is relatively rich in bat fauna comprising approximately 120 species, out of which 14 are fruit-eating and the remaining are insect-eating bats[6]. Among fruit bats, the Indian flying fox *Pteropus giganteus*, the fulvous fruit bat *Rousettus leschenaulti* and the short-nosed fruit bat *Cynopterus sphinx* are widely distributed in the Indian subcontinent.

While surveying for reptiles and mammals at the High Wavy Mountains of Tamil Nadu in southern India, Hutton[7] collected four species of bats namely *P. giganteus*, *C. sphinx*, the painted bat *Kerivoula picta*, and the yellow bat *Scotophilus heathii*. He collected only one of the several *C. sphinx* captured, presuming that it was not uncommon in India, and deposited it in the Bombay Natural History Society (BNHS), Mumbai. More than two decades later when working on the specimens of megachiropterans at BNHS, Thonglongya[8] noticed that the specimen labelled *C. sphinx*, collected at the High Wavy Mountains, was wrongly identified. He identified it as a new genus *Latidens* and named the species *salimalii*, after India's eminent ornithologist Salim Ali[8]. *Latidens salimalii* Thonglongya, 1972 is endemic to south India. The International Union for Conservation of Nature and Natural Resources (IUCN) listed this bat as 'critically endangered' and remarked as threatened by small distribution area, decline of habitat and small population size[9]. On 8 April 1993, a team from BNHS and Harrison Zoological Museum rediscovered *L. salimalii* at the High Wavy Tea and Coffee Estates (Kardana Coffee Estate) and suggested that it is the only habitat harbouring this endemic bat[6,10,11]. Recently, it entered the *Guinness Book of World Records* as one of the three rarest bats in the world[10]. Since the habitat of *L. salimalii* is highly cryptic, a study related to its ecology or ethology has not been made so far. We made an attempt to capture this enigmatic species by setting mist nets during night hours in the estate area.

The High Wavy Mountains are situated near Chinnamanur, about 70 km away from Madurai (9°58′N, 78°10′E) towards the west. Kardana Coffee Estate is situated in the broad-leaved montane forest, interspersed with coffee bushes, at an altitude of about 460 m above mean sea level[6].

Netting sessions were carried out on 24 July and 17 August 2002 during entire nights between 1830 and 0430 h, for a total of 51 net hours. Three mist nets