DNA barcoding for identification of the enigmatic plant *Ramkand*

For several years, the exact identity of the *Ramkand* had been a curiosity for plant researchers and students. Even though the so-called *kandmool* or tuber is being sold for several years at various places, especially at places of pilgrimage, its source is one of the best kept secrets by its vendors. The name and information provided by the vendors give an impression, that the tubers were eaten by Lord Rama during his days of exile.

The efforts made by several workers to identify the plant proved unsuccessful. The only material available for study is the slice which is sold. Anatomical study shows typical monocotyledonous vascular bundle arrangement. But this only added to the confusion, as monocots have adventitious roots and not a tap root system.

Therefore to find out the source, the plant material was obtained from one of the vendors from Jyothiba hill temple at Wadi Ratnagiri, Kolhapur District, Maharashtra. Slices of approximately 4.5 inches radius and 2–3 mm breadth were purchased and brought to the laboratory. DNA was extracted from these slices using the protocol described by Doyle and Doyle[1] and its purity was checked on agarose gel. For identification of the plant species, the plastid locus for maturase *k* (*matk*) was selected. The plastid and mitochondrial DNA have been viewed as the most appropriate regions to sequence for species identification in plants and animals respectively[2]. The amplification conditions included 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 s, and extension at 72°C for 50 s, with an initial 1 min denaturation step at 94°C and final extension at 72°C for 5 min. Partial amplification of the gene *matk* was achieved and the 1 kb amplicon was sequenced. The sequence obtained was edited and submitted to EMBL (accession no. FR717534). This sequence was used to find similarity with other submitted sequences. The similarity search showed 89% identity with the partial sequence of the plastid locus maturase of *Agave sisalana*. Further to confirm the identification, plants of *Agave* were visited and their leaves enclosing the rosette and juvenile inflorescence were excised, which exposed the core of the rosette. This core is soft and of similar dimension to that of the *Ramkand* being sold (Figure 1). Even though the source of obtained plant material was identified as *A. sisalana* on the basis of percentage of identity, it is possible that other species of *Agave* are also being processed and used for the same purpose. It is obvious that there are several factors in the identification of a species, but getting the field of possibilities narrowed to this extent can also help in identification of such cryptic plants.

The potential use of DNA barcodes for plant taxonomists is to elucidate species limits. The term 'DNA barcode' gives the impression that each species is differentiated by a unique sequence, but there is considerable genetic variation within each species as well as between species. However, the variations within a species are usually less than those between species. This assumption forms the premise of the above-mentioned method of identification. This assumption gave 98–100% species identification success rate[3,4]. Development of a system based on one or two plastid DNA regions in land plants has been advocated to facilitate the need of the wider scientific community for rapid and reasonably accurate identification of unknown species[2].

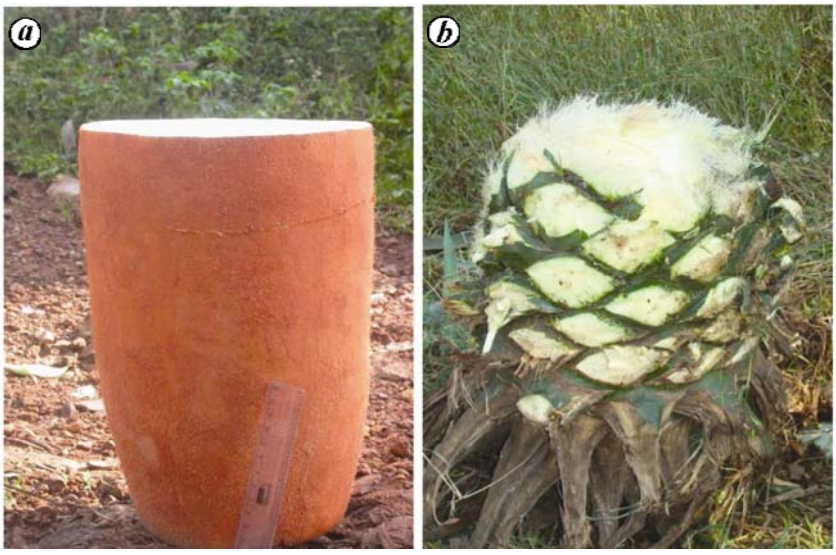

Figure 1. ***a***, The *Ramkand*; ***b***, The excised rosette of *Agave sisalana*.

1. Doyle, J. J. and Doyle, J. L., *Focus*, 1990, **12**, 13–15.
2. Chase, M. W., Salamin, N., Wilkinson, M., Dunwell, J. M., Kesanakurthi, R. P., Haider, N. and Savolainen, V., *Philos. Trans. R. Soc. London, Ser. B*, 2005, **360**, 1889–1895.
3. Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. and Hallwachs, W., *Proc. Natl. Acad. Sci. USA*, 2004a, **101**, 14812–14817.
4. Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S. and Francis, C. M., *PLoS Biol.*, 2004b, **2**, 1657–1663.

ACKNOWLEDGEMENTS. We thank the Head, Department of Botany, Shivaji University, Kolhapur for providing the facilities and Yashwant Chavan, Genome Biotech, Pune for help.

MANSINGRAJ S. NIMBALKAR[1]
PARTHRAJ R. KSHIRSAGAR[1]
VINOD B. SHIMPALE[1]
NILESH V. PAWAR[1]
GHANASHAM B. DIXIT[2,*]

[1]*Sahyadri Group Enriching Nature, Environment and Science, Kawla Naka, Kolhapur 416 003, India*
[2]*Department of Botany, Shivaji University, Kolhapur 416 004, India*
**e-mail: g_b_dixit@yahoo.co.in*