

( $P < 0.05$ ) lower in 1996 as compared to those in 1992. The other most significant change observed was that the incidence of oligospermia increased from 25% in 1992 to 35% in 1996 (Table 1). There was no significant change in the incidence of azoospermia, asthenospermia, or teratospermia.

There was good inverse correlation between the ambient SPM concentrations on one hand and semen volume and sperm concentration on the other ( $r = -0.8193$  and  $-0.83087$  respectively for semen volume and sperm concentration vs SPM). A similar correlation could not be discerned between semen characteristics and ambient levels of sulphur dioxide and lead.

The results of this retrospective study, which indicate a decline in semen quality during the five years of study, are similar to those made in USA and Europe<sup>3-10</sup>. A significant finding is the inverse correlation between the mean semen volume and sperm concentration on one hand and SPM on the other as well as the rising incidence of oligospermia with a progressive deterioration of the environment. Bangalore is one of the fastest growing cities of Asia with a concomitant rise in the human population, motorized vehicular traffic and in the increased use of diesel generators—all of these contribute to the rise in total air pollution levels in

the city. In addition to SPM, the organic and inorganic material that bind to it are substances well known to have detrimental effects on spermatogenesis and accessory sex glands.

The present study is far from comprehensive. Nevertheless, it clearly shows that semen volume, sperm concentrations and the incidence of oligospermia have drastically changed in such a manner that is indicative of reduced male fertility during the last five years. These findings give cause for concern about the altered reproductive health status of men *vis-à-vis* deterioration of environmental conditions. Spermatozoa are single cells and are likely to be the most vulnerable cells in the body to altered environmental conditions. If men are thus affected, surely women too would be similarly influenced by the environment. There is therefore an urgent need to carry out an in depth study of declining reproductive health parameters *pari passu* with environmental degradation. Such studies are underway in our Centre.

1. Schragg, S. D. and Dixon, R. L., *Annu. Rev. Pharmacol. Toxicol.*, 1985, 25, 567-592.
2. Xuezhai, J., Youxin, L. and Yilan, W., *Biomed. Environ. Sci.*, 1982, 5, 266-275.
3. Auger, J., Kunstmann, J. M., Czyglik, F.

and Jouannet, P., *N. Engl. J. Med.*, 1995, 332, 281-285.

4. Bujan, L., Mansat, A., Pontonnier, F. and Mieusset, R., *BMJ*, 1996, 312, 471-472.
5. Adamopoulos, D. A., Pappa, A., Nicopoulou, S. *et al.*, *Hum. Reprod.*, 1996, 11, 1936-1941.
6. Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N. E., *BMJ*, 1992, 305, 609-613.
7. Ginsburg, J., Okola, S., Prelivic, G. and Hardiman, P., *Lancet*, 1994, 343, 230.
8. Giwercman, A., *Hum. Reprod.*, 1995, 10, Suppl. 1, 158-164.
9. Van Waelegheem, K., DeClercq, N. and Vermeulen, L., *Hum. Reprod.*, 1996, 11, 325-329.
10. Irvine, D. S., Cawood, E., Richardson, D., MacDonald, E. and Aitken, J., *BMJ*, 1996, 312, 467-471.
11. Sharpe, R. M. and Skakkebaek, N. E., *Lancet*, 1993, 341, 1392-1395.
12. Cotton, P., *JAMA*, 1994, 271, 414-415.
13. World Health Organization, *Laboratory Manual for the Examination of Human Semen and Semen Cervical Mucus Interaction*, 3rd edn, Cambridge University Press, 1992.

RAJVI H. MEHTA  
T. C. ANAND KUMAR

*Reproductive Health Clinic and  
Research Centre,  
12, Aga Abbas Ali Road,  
Bangalore 560 042, India*

## ***Bambusa vulgaris* blooms, a leap towards extinction?**

*BAMBUSA vulgaris*<sup>1</sup> belonging to the tribe Bambuseae of Poaceae is the most widely grown bamboo throughout the tropics. Though described in 1810, the origin and nativity of this species is still debated<sup>2-5</sup>, and it survives only in cultivation. Adaptability to different agroclimatic conditions, high culm strength, utility in various ways, high pulping quality, easy response to vegetative propagation, vigorous growth, quick recovery of clumps after felling and rare flowering are some advantages of this species.

The incidence of flowering in this species is very rarely reported. Blooming occurred in Bangladesh during 1851 and 1879, in Sri Lanka in 1863, India (Calcutta) in 1890 and Singapore in 1892 (refs 2, 6, 7). After the lapse of over a

century, another flowering was reported in Bangladesh<sup>8,9</sup> during 1979, 1980-81 and 1983-84. Though not clearly indicated, Soderstrom and Ellis<sup>4</sup> studied flowering samples of *B. vulgaris* that flowered in Kandy district of Sri Lanka as late as 1970s. The flowering cycle is believed to be 80 ( $\pm 8$ ) years<sup>10</sup>. During each occurrence of flowering only a few clumps were involved<sup>3,8,11</sup> and no report of gregarious flowering exists. Unusually, flowering was not followed by fruit setting in any recorded history and eventually clumps involved perished<sup>3,8,10</sup>. Banik<sup>10</sup>, however, reported a clump which 'stopped flowering and revived'. Except a doubtful report by Lantican *et al.*<sup>12</sup> seeds of *B. vulgaris* remain to be botanically known<sup>2-5</sup>. As flowering does not

result in fruit setting, it was subsequently doubted<sup>8,9</sup> whether this condition will lead to the eventual extinction of this unique species.

We observed, in May 1996, five clumps of this species in flowering in two private areas near the police station, Cherthala (9.42°N, 76.2°E), Alappuzha district in Kerala. (*Exsiccate*: 24 May 1996, K. C. Koshy 28668, 28669; TBGT). Incidentally, this is the report of its flowering from India after 100 years. Out of the five flowered clumps, culms in four were yellow with green stripes and the other with culms yellow (with green stripes) and green (with yellow stripes) together. Two clumps in one area were completely leafless and in full bloom (complete flowering<sup>10</sup>). No new shoots were produced from these clumps.



The efforts to find out fruits and seedlings were also futile.

In the other nearby area, 3 part-flowering<sup>10</sup> clumps were noticed. One clump was healthy and new sprouts were produced (culm sheaths were available). Flowers were produced only from branch complements up to 2–3 nodes above ground. In the other two clumps all culms were completely felled and new shoots were being produced from the stumps. Flowers were produced from branch complements arising from lower nodes of cut culms.

On a repeated visit to the same spot on 30 October 1996 after rainy season it was found that the two completely flowered clumps were felled. No seedlings could be located from the surrounding

grounds. The other three partly-flowered clumps were healthy and produced new shoots. Flowers were seen on branch complements of old culms. To facilitate easy observation, offsets from part-flowering clumps were taken and planted in the bambusetum of Tropical Botanic Garden<sup>13</sup>. These offsets established in the field produced flowers for about 6 to 8 months and died without producing any seed. Artificial pollination conducted also did not yield any fruit. The florets were examined carefully to find out the possible physical barriers of sterility.

Spikelets usually contain 6 florets and a terminal rudimentary one. All florets often do not open. In many cases stamens of 3 florets come out and in other florets the sex organs do not attain

maturity and dry while remaining inside the palea.

The palea which encloses the sex organs is two keeled with wing-like margins infolded and sulcate between keels. The keels are ciliate on upper portion. The palea is closely overlapped by lemma. At the time of opening due to the pressure of well developed and massive anthers, lemma and palea widen at tip keeping a gap of 1.5–2 mm through which the stamens come out. Once the stamens emerge out, lemma and palea close back to its original position (Figure 1 a).

The gynoecium is found to be 'unhealthy' and falls under three categories. The length of entire gynoecium is (a) less than the length of palea. This is observed in majority of cases; (b) as long as palea



Figure 1 a, b. *Bambusa vulgaris* in bloom. a, Close view of one spikelet. The hairs on the keels of palea and the opening of florets are seen ( $\times 6$ ). b, A portion of flowering branch showing clusters of spikelets and opened florets ( $\times 2.25$ ).



or (c) slightly longer (middle stigma only) than lemma as in well developed ones. Only in such cases at least a portion of stigma comes out on opening of floret.

The stigma is usually two in number, the third one is not well developed. The entire stigmatic portion is only 3–4 mm long. Tip of middle stigma of 1.5–2 mm struggles, along with stamens, to come out when lemma and palea widen. Rest of stigma remains inside the closed floret. The receptive stigmatic portion exposed to receive pollen is thus very limited.

The bristle-like hairs of palea (Figure 1a) are longer than the stigmatic hairs and in an opened floret the exposed but small stigmatic portion is often covered (?) by the hairs on the two keels of palea. Possibly this can act as a barrier preventing stigma from receiving the pollen grains.

Moreover, the reported<sup>10</sup> higher percentage of sterility (70–92%) of pollen may also contribute to the sterility in *B. vulgaris*.

The available evidence points to the imminent danger of extinction of this mysterious species due to (i) the death of clumps after flowering, (ii) the lack of fruit set, (iii) the inherent 'unhealthy'

nature of stigma to receive pollen, (iv) the possible role of bristle-like hairs as barriers preventing pollination, and (v) the high pollen sterility.

1. Schrader ex Wendland, *Collect. Pl.* 1810, p. 26, pl. 27.
2. Gamble, J. S., *The Bambuseae of British India*, Ann. Roy. Bot. Garden, Calcutta, 1896, vol. 7, pp. 43–45, pl. 40.
3. McClure, F. A., *The Bamboos, A Fresh Perspective*, Harvard University Press, Cambridge, 1966.
4. Soderstrom, T. R. and Ellis, R. P., *The Woody Bamboos (Poaceae: Bambuseae) of Sri Lanka: A Morphological Anatomical Study*, Smithsonian Institution Press, Washington DC, 1988, pp. 39–45.
5. Dransfield, S. and Widjaja, E. A., *Plant Resources of South East Asia, No. 7, Bamboos*, Backbuys Publishers, Leiden, 1995, pp. 74–78.
6. Bennet, S. S. R. and Gaur, R. C., *Thirtyseven Bamboos Growing in India*, Controller of Publications, Delhi, 1990, pp. 43–44.
7. Tewari, D. N., *A Monograph on Bamboo*, International Book Distributors, Dehra Dun, 1992, pp. 51–53.
8. Banik, R. L., *Bano Biggyan Patrika*, 1979, 8, 90–91.
9. Banik, R. L., in *Recent Research on Bam-*

*boos* (eds Rao, A. N. et al.), IDRC, Canada, 1987, pp. 160–169.

10. Banik, R. L., in *Bamboo and Rattan Genetic Resources and Use* (eds Rao, V. R. and Rao, A. N.), International Plant Genetic Resources Institute, Italy, 1995, pp. 1–22.
11. Hildebrand, F. H., *Indonesia Forest Res. Inst. Rep.*, 1954, 66, 82.
12. Lantican, C. B., Palion, A. M. and Saludo, C. G., in *Recent Research on Bamboos* (eds Rao, A. N. et al.), IDRC, Canada, 1987, p. 52.
13. Koshy, K. C., in *Proceedings of Symposium on Rare, Endangered and Endemic Plants of the Western Ghats*, 1991, Kerala Forest Department, Thiruvananthapuram, 1993, pp. 174–180.

ACKNOWLEDGEMENTS. We thank Planning and Economic Affairs Department, Government of Kerala for financial assistance under Western Ghats Development Programme and Mr C. Suseendran for Photography.

K. C. KOSHY  
P. PUSHPANGADAN

*Tropical Botanic Garden and  
Research Institute, Palode,  
Thiruvananthapuram 695 562, India*

## Restriction fragment length polymorphisms of the rRNA genes in some pulses

Recognition and exploitation of variations among genetically divergent groups of germplasm are fundamental in breeding and genetic engineering programmes. Restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), DNA finger printing, inter simple sequence repeat amplification (ISSR) and amplified fragment length polymorphisms (AFLPs) are powerful tools for studies of plant genetics, evolution, germplasm diagnosis and crop improvement<sup>1–4</sup>. These techniques allow a direct analysis of the plant genome at the DNA level. RFLP analyses have been used as molecular markers to construct linkage maps of crop plants, to mark quantitative trait loci and to complement phylogenetic relationships in

several plant taxa<sup>5–7</sup>. rRNA genes, although not with the same impact as chloroplast DNA, have proven to be of tremendous utility in phylogenetic reconstruction<sup>8</sup>. They also provide valuable genetic markers for the analysis of genomic relationships among cultivated species and their wild relatives<sup>9</sup>. The tandem arrays of rDNA repeat units, generally located in the nucleolar organizing regions (NORs) of chromosomes, combine highly conserved gene regions, encoding rRNA, with more variable intergenic spacer regions (IGS). IGS regions, which separate the adjacent transcription units, have been found highly variable in sequence, length and copy number of subrepeats in several plant genera<sup>10</sup>. Because rRNA gene

sequences are subjected to relatively rapid rates of concerted evolution<sup>11</sup>, they produce DNA fragmentation patterns that are highly homogeneous within individuals and among closely related populations or species, yet exhibit characteristic heterogeneity between groups. In comparison, single copy gene markers<sup>12</sup> often tend to exhibit as much within-group as between-group variation in plant species. rRNA polymorphisms can, therefore, constitute useful genetic markers<sup>9,13</sup>. To achieve a better understanding and to provide molecular evidence for the systematic relationship between and within some populations of *Lablab*, *Dolichos* and *Vigna* species we investigated the polymorphism of the rRNA genes.

Five populations of *Lablab purpureus*,