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Bamboos – Some newer perspectives

C. K. John, Rajani S. Nadgauda and A. F. Mascarenhas

Bamboos are a part of the life and culture of southeast Asian people. They are a sought-after industrial raw material of considerable economic importance. All possible methods for their optimum utilization need to be tried. Bamboos have a peculiar behaviour of flowering and seeding at the end of very long vegetative growth phases, the length of which is considered to be species-specific. This makes their perennial propagation by seeds and their improvement by hybridizations practically impossible. Some inherent properties of bamboos can be exploited profitably to overcome these difficulties. In vitro induction of flowering has vast potential in bamboos. Bamboos need to be viewed in a broader perspective.

BAMBOOS are a most useful group of plants, and are members of the grass family (*Poaceae*)[1]. Two important characters which make (majority of) them distinct from other grasses are: (i) woody perennial habit and (ii) peculiar flowering and seeding behaviour[2]. Most woody bamboos flower and seed at the end of very long periods of vegetative growth[3] (Figure 1). In India, bamboos are the major source of raw material for pulp and paper industries[4]. Besides, they are also used for a variety of

The authors are in the Division of Plant Tissue Culture, National Chemical Laboratory, Pune 411 008, India

purposes like house construction, making of household utensils, agricultural implements, handicrafts and over a thousand other uses[5,6].

The demand for bamboos is increasing much more than their availability. In the near future, a major shortfall in the availability of bamboo raw material for the paper industry is expected[7]. Due to severe deforestation and the present limitations of bamboo propagation and improvement, it is unlikely that the increasing demand will be fulfilled. This emphasizes the need for enhancing bamboo production. This can be achieved by (i) increasing the area under bamboo cultivation, (ii) selective multiplication of better clones and (iii) use of improved varieties.

Bamboos are well known for their fast growth, and their use as a substitute for timber would greatly reduce deforestation. Bamboos need to be given more importance in agro-forestry, social forestry and wasteland develop-

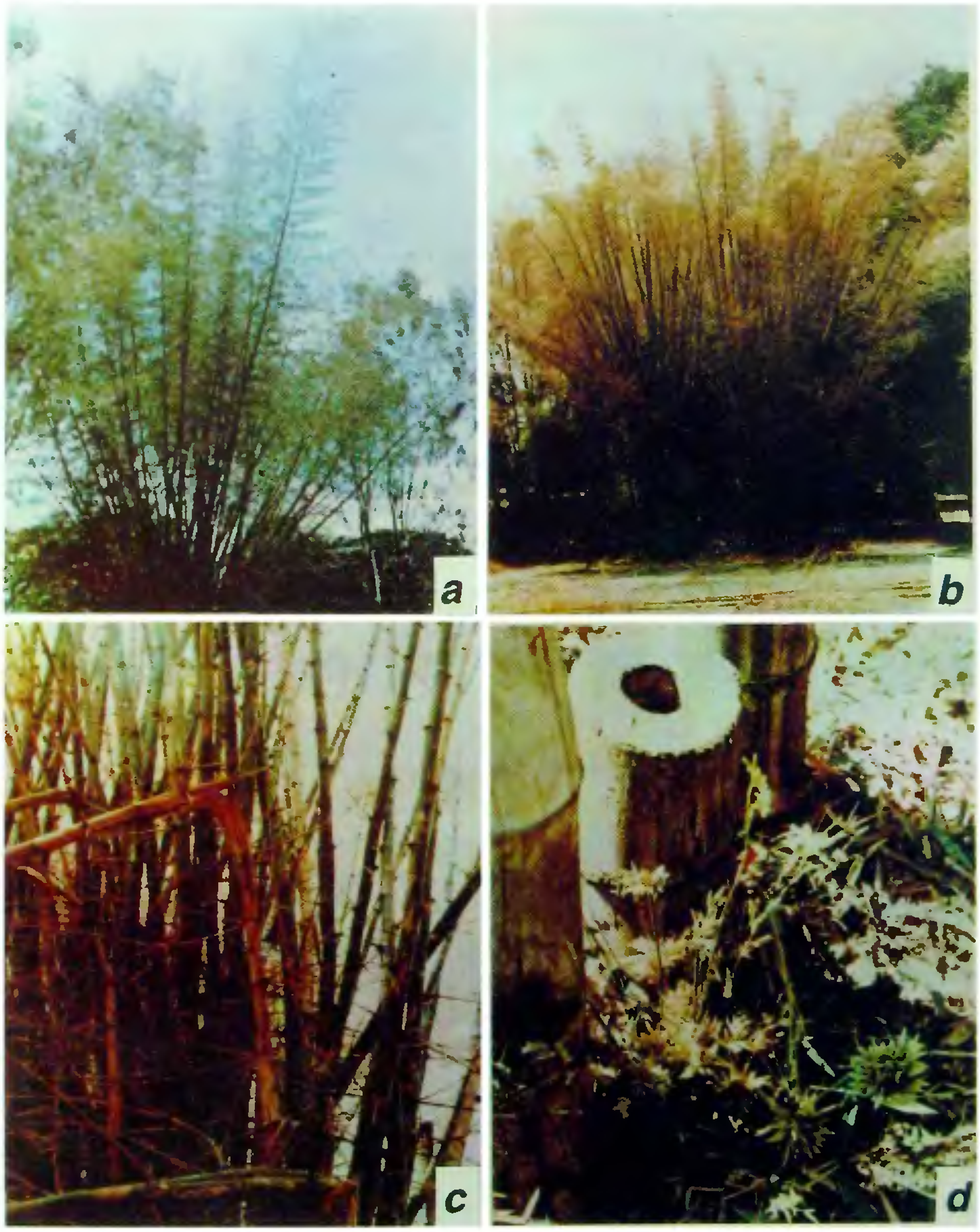

Figure 1. Sequence of events during flowering, seeding and death of a bamboo clump *a*, one clump in vegetative phase. *b*, a flowering and seeding clump; *c*, death of a flowered clump; and *d*, coppicing and flowering after the culms are cut in *Bambusa arundinacea*.

ment. Rural economies in southeast Asian countries are dependent on biomass production and popularization of bamboo cultivation, and bamboo-based industries would greatly help in rural development.

The potentials of bamboos are well understood and efforts are being made to overcome the difficulties in their propagation and improvement. In recent years tissue culture methods have also been standardized for their propagation[8–17]. One of the outstanding achievements in this field is the *in vitro* induction of flowering in bamboos within a few months as against many years in nature[18–20].

In this article we attempt to view the potentials, problems and prospects of bamboos in a broader perspective.

The peculiar flowering behaviour of bamboos

Brandis[21] classified bamboos into three categories on the basis of their flowering behaviour: (i) species which flower annually (or nearly so), (ii) species which flower gregariously and periodically and (iii) species which flower irregularly. According to Blatter[22], these three categories are fairly complete. Most of the woody bamboos belong to the second category, in which the intermast periods range between 3 years (in *Schizostachyum elegantissimum*) and 120 years (in *Phyllostachys bambusoides*)[3]. *Bambusa vulgaris* (syn. *B. striata*) is considered either as a sterile mutant or as having an intermast period of 150 years (or more).

Though much is written about the species-specific, supra-annual intermast periods, actual data on them is very little. From the published flowering dates and intermast periods, it appears to be not very rigid. For example, in *Phyllostachys bambusoides* gregarious flowerings were reported in 999 and 1114 AD (intermast period 115 years), 1716–1735, 1844–1847 and 1966–1969 (intermast periods?), though this species is considered as having a flowering cycle of 120 years[3]. In *Melocanna bambusoides* flowering was reported in 1863–1866, 1892–1893, 1900–1902, 1933 and 1960 from Mizoram hills (intermast periods 26–30 years, 7–10 years, 31–33 years and 27 years), in 1864 and 1911–1912 from Lashai Hills (intermast period 47–48 years) and in 1863–1866, 1908–1912, 1958–1959 from Chittagong, Bangladesh (intermast periods 47–48 years, 42–49 years and 46–51 years)[3]. From reports of gregarious flowering from different places intermast periods estimated for *Bambusa arundinacea* and *Dendrocalamus strictus* (the two most common bamboos of peninsular India) are 30–45 years and 20–60 years, respectively. Many bamboo species having long intermast periods are reported to flower almost every year in Thailand. These variations may be due to the existence of many cohorts having differing intermast periods or due to environmental (edaphic?) factors. Is the species-specific intermast period constant? To understand this the lengths of the intermast period are to be studied in detail in large number of species. When variations do exist, their extent needs to be assessed. This would establish whether flowering in bamboos is under the sole control of an internal calendar (genetic control), totally free from environmental factors, or there is any interaction between the internal clock and the environment.

When does the countdown of the intermast period start? Is it from seed or seedling?[23] Seeds, until germination, are almost inert – metabolically. Probably, the countdown may be from seedling. This can be verified by storing seeds (under appropriate conditions), raising plantations at time intervals (6 months, 1 year, etc.) and checking whether all of them flower at the same time, or they show the same periodicity at which they are raised from the seeds. These can be studied only in species having shorter intermast periods. An attempt made in *Ochlandra ebracteata* (intermast period about 7 years) was not successful. The berry-like seeds (fruits) in this species are highly recalcitrant and lose viability very fast. A wide variety of storage conditions tried were not suitable for prolonging seed viability in this species.

When a species (cohort) flowers gregariously, few individuals flower one year earlier (preceding tail) or later (succeeding tail). Thus, flowering and seeding occur in three consecutive years.

Some of the gregariously and periodically flowering bamboos also flower sporadically at irregular intervals[1]. Sporadic flowering occurs in isolated clumps, in few culms of a clump or in few branches of a culm, producing very little or no seeds[24]. In *B. arundinacea* and *D. strictus* flowering is encountered almost every alternate year in some places[25]. When there is seed-set, sporadic flowering also is a source of seeds during long intermast periods. Studies on the reproductive biology of bamboos are needed to understand the reasons for reduction/absence of seed-set in sporadic flowering.

Reproductive biology in bamboos

There are very few reports on the reproductive biology of bamboos, possibly because of their flowering only at long intervals. Some interesting observations were made from studies on reproductive biology in three species of bamboo, *B. arundinacea*, *D. strictus* and *Melocanna bambusoides*[26–28] (Figure 2). Bamboos can be divided into two categories on the basis of maturing of the reproductive structures (androecium and gynoecium): (i) species in which androecium and gynoecium mature at the same time, as in *B. arundinacea*, and (ii) species in which androecium and gynoecium mature at different times (dichogamy), as in *D. strictus*.

In the first category androecium and gynoecium remain at two different planes, anthers at a much lower plane than the stigma, acting as a barrier to self-pollination. In the second category gynoecium matures 3–4 days (or more) earlier than androecium (protogyny), preventing self-pollination.

This difference in the maturing of the reproductive structures has application in breeding. A step of emasculation can be omitted when protogynous species are used as female parents. Because of the size of the clumps (nearly 50–100 ft), and wind pollination, at the time of anther dehiscence and pollen release, self-pollen gets dispersed in the whole surrounding. In species in which androecium and gynoecium mature at the same time, maintaining fidelity of cross-pollination becomes very difficult, and protogyny can be used effectively.

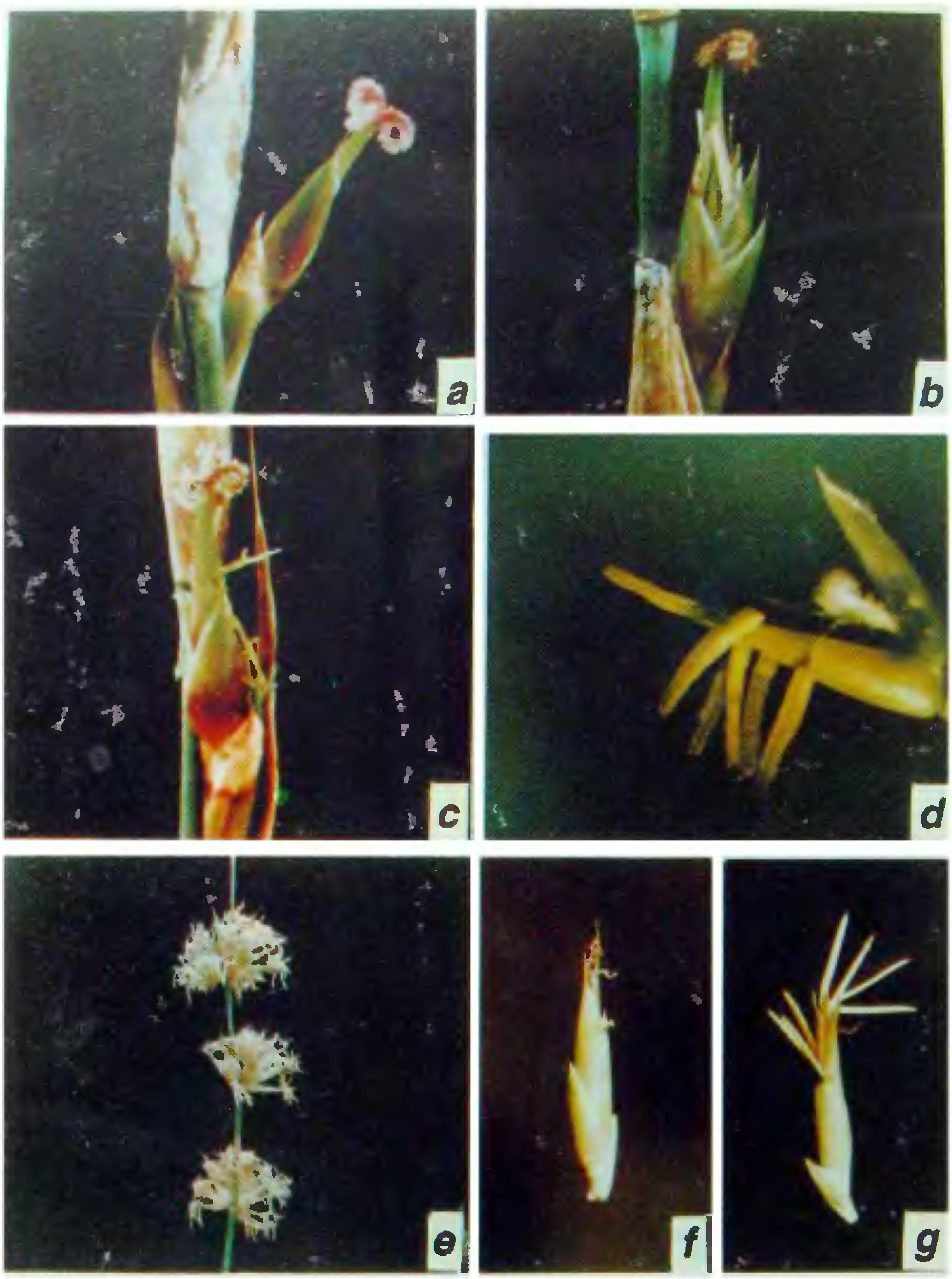

Figure 2. Bamboo florets *a*, *Melocanna bambusoides*, one floret in the female phase (note the pink, trifid stigma), *b*, a floret at the start of male phase; *c*, young anthers eaten by insects; *d*, one floret of *Bambusa arundinacea* at the time of anthesis (note the androecium and gynoecium maturing at the same time but remaining at two different planes), *e*, a spike of *Dendrocalamus strictus*; *f*, one floret in the female phase; and *g*, one floret in the male phase

Difference in the maturing of reproductive structures is of use in taxonomy. Many characters are used in dividing bamboos into major categories. Munro[29] divided them into three categories on the basis of the number of floral parts: *Triglossae*, *Bambusae verae* and *Bacciferae*. Brandis[21] divided them into three categories on the basis of flowering behaviour. McClure[1] divided them into two categories on the basis of the pattern of rhizome growth: sympodial (pachymorph) and monopodial (leptomorph). Difference in the maturing of reproductive structures can also form a character in classifying bamboos. Tribe *Bambuseae* can be divided into two categories on the basis of this character[30].

In species under the first category, the mechanism preventing self-pollination is a physical barrier and androecium and gynoecium mature at the same time. Pollen from anthers of one floret can pollinate stigmas of other florets at a lower plane either on the same culm/clump or a different culm/clump. Hence, in species under this category there can be some amount of seed-set in sporadic flowering (also in flowering in isolated clumps), provided there is no self-incompatibility, as in *B. arundinacea*. In species under the second category, since androecium and gynoecium mature at different times, the chances of seed-set in sporadic flowering are very low. Studies on sporadic flowering in *D. strictus* showed protogyny to be highly effective. Seed-set can be in the range of 0–1%, depending upon the number of culms/clumps involved and their location. The timing of anthesis (or exertion of gynoecium/androecium in species exhibiting dichogamy) is highly synchronized, and is dependent on atmospheric conditions (temperature, humidity and probably light intensity). Wind being a passive pollinator, a high degree of synchrony in the timing of anthesis (or exertion of reproductive structures) is very essential for successful pollination. Florets which are out of phase with the majority will not be pollinated. Probably, bamboos are solely wind-pollinated.

Pollination in bamboos

Bees are reported to hover around the flowers of *Bambusa polymorpha*[31]. Pollination in Chilean *Chusquea* is suggested as being assisted by wind and some small insects[32]. *B. polymorpha* and *C. abietifolia* have purple glumes and bright-yellow anthers, suggesting a role in insect pollination[3]. However, bamboos also have inflorescences/florets similar to other grasses and the florets are greatly reduced. From reports of profuse seed production when many clumps flower in close proximity, and little or no seed-set in isolated clumps (and also in sporadic flowering), it may have to be inferred that bamboos are predominantly out-crossers.

Only 1–2 florets mature each day and anthesis/exertion of reproductive structures in an inflorescence takes 1–2 weeks. In most bamboos anthers have long, slender filaments. When mature and fully exerted, anthers remain at a distance from the floret/receptive stigmas. The long, slender filaments help in pollen release when anthers move in the wind. Insects do not come in contact with anthers/receptive stigmas when they visit florets at the time of anthesis. Hence, they cannot act as pollinating agents. In *B. arundinacea*, *D. strictus* and *M. bambusoides* insects visit the florets much before anthesis and eat pollen from immature anthers by cutting open the anther wall[26–28]. There cannot be co-evolution between bamboos and insects because the former flower at long intervals and the latter have short life-cycles. The insect visitors cannot be obligatorily dependent on bamboos, and may have to be considered only as chance visitors of their florets.

Relatively smaller pollen grains of bamboos are shed in a dehydrated state. The stigmas are highly feathery and have many thin papillae. These characters are adaptations for wind pollination. Insects visit bamboo florets only when the climate is good. In many bamboos seed production takes place even in the absence of insect visitors.

Our knowledge on the reproductive biology of bamboos is still fragmentary and such studies are urgently needed in a large number of species. Often, bamboos flower in far-off, inaccessible forests, making detailed studies very difficult. This difficulty can be overcome by rhizome transplantation from flowering clumps[33].

Rhizome transplantation

Rhizomes from flowering clumps, when transplanted, produce coppices and flower. Coppices being only a few feet tall, flowering takes place at convenient heights. When transplanted near the laboratory, detailed studies can be made conveniently. This method can also be used for hybridizations, when two or more species flower at the same time at different (distant) locations[34].

Cryopreservation of pollen

Cryopreservation of bamboo pollen can be another method of overcoming the barrier placed on hybridizations by the peculiar flowering behaviour[23]. Long-term storage of pollen in many species would help in obtaining overlap in the availability of both male and female gametes in two or more species. Venkatesh[35] has reported approximately 90% germination of *Ochlandra travancorica* pollen in a 1% sucrose solution. Studies on *B. arundinacea* and *D. strictus* showed sugar specificity for pollen germination and pollen tube growth. Addition of components of Brewbaker–Kwak[36] medium at optimum concentrations also helps[26].

Since seeds in most economically important bamboos are available only at long intervals, it is essential to make their proper use.

Storage of bamboo seeds

In bamboos there are broadly two seed (fruit) types, caryopsis and berry (Figure 3). *B. arundinacea* and *D. strictus*, in which the seeds are caryopses, seed production takes place over many months. In Pune, when these species flowered in 1990–1991, flowering started in August–September. Ripe seeds started falling off from the tree by December and continued till May–June. By March–April thick carpets of ripe seeds were covering the ground below the clumps. There were many seed predators, and squirrels, rats and birds being the most common.

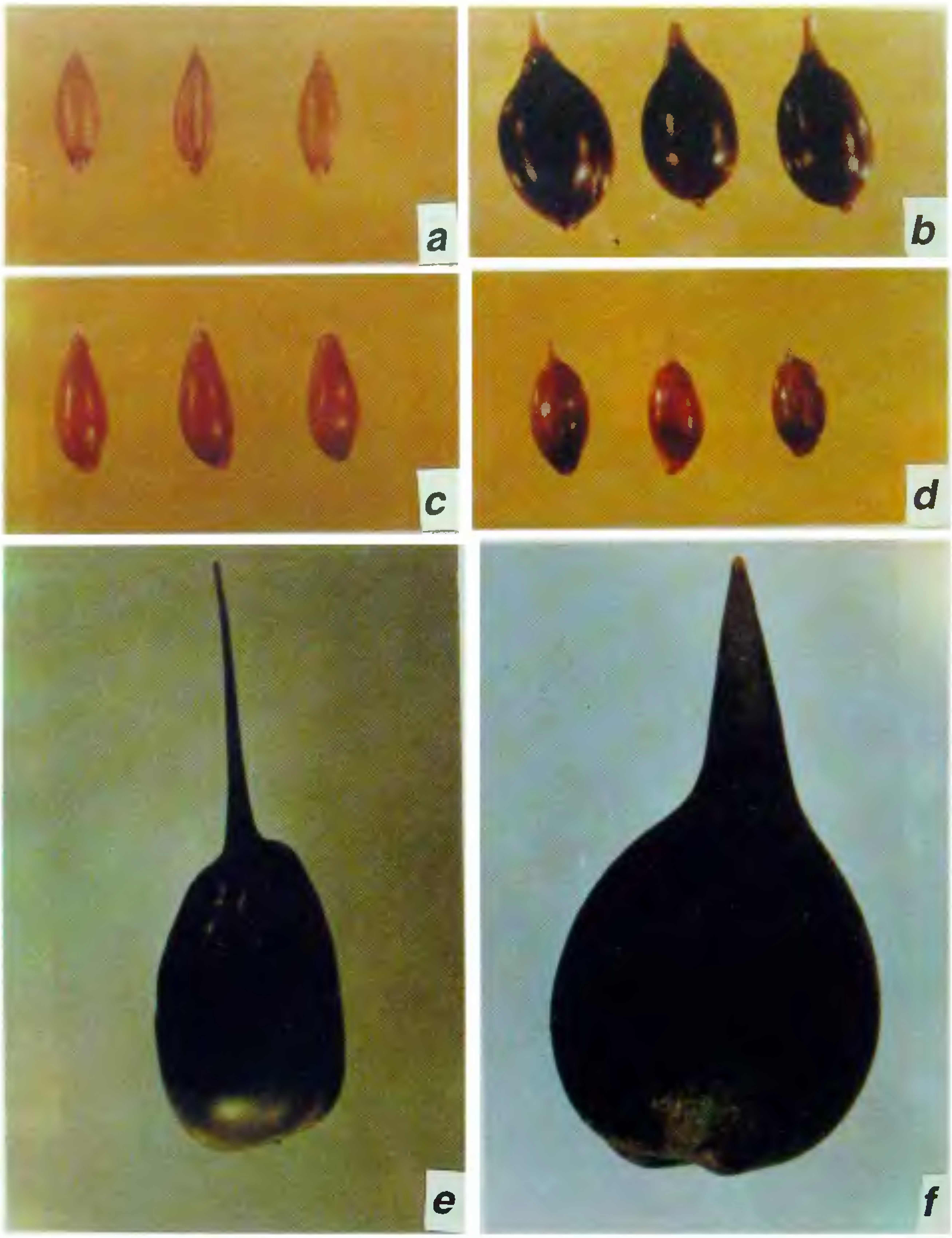

Figure 3. Bamboo seeds ***a***, *Bambusa arundinacea*, ***b***, *Cephalostachyum pergracile*; ***c***, *Dendrocalamus hamiltonii*, ***d***, *Dendrocalamus strictus*; ***e***, *Ochlandra ebracteata*, and ***f***, *Melocanna bambusoides*. In the former four species seeds are grain-like, with less moisture contents and in the latter two species they are larger and fleshy.

These seeds (caryopses) are shed in a dehydrated state. Prolonged seed viability on storage is dependent on many factors. Cleanliness of the seed lot, seed moisture content and storage temperature are very important. When seeds are stored without cleaning, loss of viability is rapid, possibly because of degradation by pathogens. Clean seeds remain viable for longer periods. When seeds are collected, cleaned, sun-dried and stored at lower temperatures (–70°C), they retain viability for long (more than three years), though there is a gradual decrease in viability. Storage of bamboo seeds for many months over dehydrants at lower temperatures is reported[37]. Seeds exposed to pre-monsoon rains also lose viability faster. Hence, it is essential that seeds are collected, cleaned, sun-dried and stored at regular intervals rather than once, by the end of summer. This would also act as a check on seed predators.

In 'berry-bearing bamboos' (species of *Melocanna*, *Melocalamus* and *Ochlandra*), the berries take longer to mature (6–9 months). Most of them mature nearly at the same time, just before or by the onset of monsoon. Vivipary is observed occasionally (Figure 4). The moisture content of the berries is very high. They lose viability as moisture is lost and cannot be stored at lower temperatures.

Standardization of long-term storage conditions of bamboo seeds would be of great value. Since cold-room facilities are now available in all major cities, forest agencies would also be able to use these methods. This would help, at least partly, in solving the problem of non-availability of seeds during the long intermast periods.

Use of stored seeds for many years for establishment of plantations would result in a breakdown of synchrony in flowering cycle in the course of time (provided the countdown of the intermast period starts from seedlings and not from seeds).

Seed orchard

In many bamboo species possibly there are many cohorts differing in the length of intermast periods (?), and the time of flowering. During gregarious flowering, seeds are available for three consecutive years in many of them. If seedlings are established from seeds from all three years, in the following generation flowering and seeding will take place in five consecutive years, and in further generations for seven years, nine years and so on. According to Janzen's[3] hypothesis, this may be due to the breakdown of the tropical forest ecosystems and absence/reduction of (majority of) seed predators from the habitats in which bamboos grow at present.

Some species of bamboo also flower sporadically at irregular intervals[24], and flowering and seeding are available every few years (at different locations). In *B. arundinacea*, *D. strictus* and some others sporadic flowering is very common[25]. Obtaining seeds of these species is not considered difficult. Prasad[38] has reported successful raising of *D. longispathus* plantations over many years from seeds obtained from sporadic flowering.

Establishing a seed orchard of bamboos would help in obtaining perennial seed supply. Many cohorts of different species can be grown at one place, as a source of seeds and for field studies on bamboos.

Why is the intermast period so long?

The question 'why is the intermast period so long?' (in bamboos) has intrigued many but addressed by very few. Janzen[3] explained it as an adaptation, to satiate the seed predators at the prey population level. The long intermast periods are considered to have developed as a result of selection. When large amounts of seeds are produced, a sufficient number are left after satiating the local and transient predators. Wind pollination prevalent in bamboos makes it essential that the intermast periods increase by doubling, so that the mutants (?) are not at a disadvantage for pollination. A survey of literature on this aspect[3] (however meager it is) shows that it may not be true. The minimum intermast period in bamboos is 3 years (in *Schizostachyum elegantissimum*). Doubling would give 6 years, 12 years, 24 years, 48 years, 96 years, etc. If most common intermast periods are considered, it can be 15 years, 30 years, 60 years, 120 years and so on. But these are not the only intermast periods. Information on intermast periods summarized by Janzen[3] also shows many odd figures.

One method implicated in the lengthening of intermast periods is polyploidy. Most of the mast-seeding bamboos are highly polyploid. However, *prima facie* there is no correlation between the ploidy levels and the lengths of intermast periods, when bamboos are taken either as a whole or when individual genera are considered. For example, in the genus *Phyllostachys*, *P. aurea*, *P. bambusoides* and *P. henonis* have chromosome numbers $2n = 48$ (possibly tetraploids)[2]. *P. aurea* has an intermast period of 15 (14–17, 13–19) years, *P. bambusoides* 120 years and *P. henonis* 60 years[3]. In the genus *Dendrocalamus*, both *D. brandisii* and *D. giganteus* have chromosome number $2n = 72$ (possibly hexaploids). Their intermast periods are about 20 years and 76 years, respectively. Many species of south Indian *Ochlandra* have chromosome numbers $2n = 72$, though they have shorter intermast periods, in the range of around 7 years.

Death of the parent

The death of the bamboo parent after flowering and seeding used to be considered as an intriguing phenomenon for long[1]. Nicholson postulated that the death of the adult bamboo removes the intense shade they cast and helps in the establishment of the seedlings[39]. In Janzen's opinion the adult bamboos die because they put in all their stored resources in producing a large seed crop (due to selection pressures). Small amounts of resources saved would not be sufficient to maintain the adult in competition with a large number of its seedlings and other species of plants and also at the face of challenges from herbivores (local and transient). Holding back enough resources would jeopardize the size of the seed crop. Moreover, if a 'monocarpic' ('semelparous' – flowering and seeding only once at the end of the vegetative growth phase) mast-seeding bamboo has to become 'iteroparous' (flowering annually for many years after attaining maturity), it would need two internal calendars, one to tell when it germinated from

Figure 4. ***a***, A maturing fruit of *Melocanna bambusoides* (note the beak, pointing downwards, which fixes the fruit on falling on ground); ***b***, viviparously germinated fruit of *Ochlandra ebracteata*, ***c***, same as in ***b***, on further growth; ***d***, one fruit of *Ochlandra stridula*, an iteroparous species; ***e***, an albino seedling of *Melocanna bambusoides*, ***f***, flowering and fruiting in coppice developed from a transplanted rhizome of *Melocanna bambusoides*, and ***g***, maturing fruit on a coppice.

the seed and the other one to tell when it flowered last[3]. If bamboos are to become iteroparous, they may not need two calendars. They would flower every year on attaining maturity (under environmental control), after a certain juvenile phase. There are few iteroparous bamboos (*Arundinaria wightiana*, *Bambusa forbesii*, *Ochlandra stridula*, etc.). Bamboos would need two internal calendars, when the parent is not to die after flowering but is to flower after growing vegetatively for another round of its species-specific intermast period, and repeat it many times.

Monocarpy is very common among members of the grass family[40]. Most of them are herbaceous seasonals or annuals. In spite of its huge size, the grass family is a coherent one, exhibiting characteristic combinations of unusual morphological and anatomical features. They have high capacity for hybridization and polyploidy. Their morphology, anatomy, habitat and reproductive cycles contribute to their competitive success and versatility[41]. Bamboos are arborescent and grow as thick forests, conspicuous because of their size. Culms are the useful plant parts (unlike seeds in cereals). After (gregarious) flowering and seeding, whole bamboo forests which existed for many years get wiped out. These may be the reasons for their death getting more attention[40].

Propagation methods

Bamboos are propagated by seeds (when available), and a variety of vegetative propagation methods which are in use for a long time[24]. In recent years tissue culture methods have increasingly been used for their propagation[8–17].

Seeds

Seeds are the most convenient mode of propagation. Their smaller sizes allow easy transportation, and their metabolic inertness permits waiting periods of few weeks to even few months in transportation and planting. However, the peculiar flowering and seeding behaviour of bamboos makes this only of limited use in perennial raising of plantations in many species. Once methods are standardized for long-term storage of seeds, this method would be of much value.

Conventional methods

Conventional methods for vegetative propagation of bamboos are seedling multiplication, offset and clump division, rhizome cuttings, layering, culm cuttings, pre-rooted and pre-rhizomed branch cuttings, and branch cuttings and nodal bud chips[24]. Most of these methods have some disadvantages. The propagules are larger, difficult to extract, transport and plant. Their non-availability in sufficient numbers is another limitation. Extraction of vegetative propagules results in decrease in the productivity of parent clumps. Lastly, they also flower at the same time as their parents, resulting in lower total yields. One advantage is that they establish and reach the productive phase faster, 3–5 years earlier than seed-raised plantations.

Seedling multiplication does not involve many of the difficulties associated with other conventional methods of vegetative propagation. But the availability of seeds only at long intervals (not in all species), lack of methods for their long-term storage and the length of time taken to establish and reach productive phase are some of the problems encountered. At the age of 30–40 days, seedlings produce culms and develop rhizomes. By 9 months they attain a 4–5-culm stage and can be divided into three units, each having rhizome, roots and shoots. By this method the seedlings can be multiplied for few years to produce a large number of propagules.

Tissue culture methods

Most tissue culture methods are based on micropropagation and somatic embryogenesis from seedling rhizome, node and basal node explants. Somatic embryogenesis, in explants derived from inflorescences, immature and mature embryos, seedling root and seedling sheaths, is also used[10–15]. Very recently, methods for large-scale propagation of bamboos using nodal bud explants derived from mature clumps (of different ages) have also been reported[16,17]. The use of explants from mature clumps has the advantage of permitting selection for 'plus' clumps. A possible disadvantage is that being a vegetative propagation method, the plantlets may also flower along with their mother clumps. The total yield would depend on the time left for flowering. In species having shorter intermast periods (15–30 years) this can be a serious handicap. However, for species having longer intermast periods (60–120 years) this may be acceptable. Tissue culture methods allow the production of a large number of plantlets identical to the mother trees. They are smaller and can be easily transported and planted.

Selection

Many bamboo species are known to be highly heterozygous in nature[42–44]. Like grasses in general, bamboos are wind-pollinated. They have physical/physiological barriers to self-pollination[26–28,35]. These factors contribute to the high degree of genetic variability and provide ample scope for selection[45]. Since culms are the produce of economic value, increased biomass production is the main objective of selection. Faster formation of rhizomes and clumps, non-congestion of clumps, higher rates of growth and culm production, diameter of the culms, thickness of the culm walls, length of internodes, etc., are characters which contribute to higher biomass yields. Fibre length can be one important character for species used as raw material in paper industry. Disease, insect and pest resistance can also add to economic gains.

Most characters contributing to increased biomass production are expressed at the adult stage. Practising selection at the adult stage has the disadvantage of being both space- and labour-intensive. Propagation from adult clumps also results in decrease in the vegetative growth phase and total yield. Great diversity in vigour, growth habit and ultimate stature of bamboo seedlings is reported[1,46]. Folding of the first leaf towards the left or right has been found to occur nearly in the same proportion in the seedlings and is suggested as a marker for faster growth (left-handed seedlings are fast growers)[47]. Identifying additional markers, at the seedling stage, for faster growth and increased biomass production would be highly beneficial. In *B. arundinacea*, *D. strictus* and other species in which seed availability is not a serious problem, seedling selection prior to micropropagation is in practice. Modern methods of molecular biology would also be helpful in this[48,49]. Selective multiplication of better seedlings by appropriate methods and use in plantations would result in considerable increase in bamboo production. Since bamboos grow for many years before flowering, seeding and dying, the benefit of one round of selection can be reaped for many years. Depending upon the species, it would be after many years (3–120 years) that flowering and seeding result in segregation of alleles (contributing to superior traits) and their reassortment, necessitating a fresh round of selection.

Possible advantages of somatic embryogenesis

Resetting of the internal calendar takes place after sexual reproduction (seeding). Rejuvenation by embryogenesis may be resulting in resetting of the internal clock. If a similar resetting takes place in rejuvenation by somatic embryogenesis, it would be of immense value in bamboos[23,45]. Standardization of methods for somatic embryogenesis from mature explants would help in rapid, large-scale propagation of high-yielding 'plus' trees, possibly without any loss of vegetative growth phase.

In vitro induction of flowering

There are three reports on *in vitro* induction of flowering in bamboos[18–20], two dealing with induction of flowering in seedling coleoptile and node explants (in the former seeding is also reported) and one on observation of flowers in cultures derived from compact callus with embryos. So far, *in vitro* induction of flowering has been obtained in four species, *B. arundinacea*, *D. brandisii*, *D. hamiltonii* and *D. strictus*.

There are some striking similarities: (i) use of seed/seedling (juvenile tissue) as the starting material and (ii) use of BA/BAP (benzyl adenine/6-benzyl aminopurine) – a cytokinin.

Since flowering can now be obtained by *in vitro* methods in a few months, the question is: Of what use is it? The immediate answer is that *in vitro* induction of flowering is useful in perennial seed production and in hybridizations (Figure 5).

Another pertinent question is: What are the problems and prospects of using *in vitro* induction of flowering for perennial seed production and hybridizations? Firstly, flowering *in vitro* so far could be obtained only in seedling explants. This definitely is a serious limitation in the face of availability of seeds only at long intervals and their short viability. If flowering can be induced in mature explants by tissue culture methods, it will have tremendous application. Is the determination to grow for a species-specific supra-annual interval before flowering and seeding absent at the juvenile phase? (probably it sets in only after a certain period of growth). It is interesting to note that thornyness, a character of mature clumps of *B. arundinacea*, is expressed only at the age of 2–3 years or more.

Secondly, what precisely is (are) the factor(s) responsible for induction of flowering *in vitro*? Is it the cytokinin? Is it (are they) factor(s) specific to the medium? Is it a physical factor? Or, is it a combination of all these? This is a very complex problem. Is (are) the factor(s) effective *in vivo* (in field-grown plants)?

Thirdly, what are the factors controlling seeding under *in vitro* conditions? The conditions essential for seeding are: (i) production of viable gametes, (ii) pollination, (iii) fertilization and (iv) proper development of embryo and endosperm. It is observed that only around one-third of the pollen grains in *in vitro* developed florets are fertile, and there are wide variations among cultures (of the same species). Refinement of the culture conditions (chemical and physical) is necessary for increasing gamete viability. In nature, bamboos are wind-pollinated, and wind is necessary for pollination. In *in vitro* cultures a micro air current is generated inside the shake flasks, which assists pollination. In static cultures the chances of seed production are much less. In *B. arundinacea* androecium and gynoecium mature at the same time. But they remain at two different planes. Species of *Dendrocalamus* exhibit dichogamy (protogyny)[26]. It is observed that the stigmas remain fresh for longer periods under *in vitro* conditions (possibly because of near saturation of humidity), rendering dichogamy practically ineffective. Once pollination takes place, the factor affecting fertilization is the presence/absence of self-incompatibility. In *B. arundinacea* experimental studies have shown that there is no self-incompatibility[28, 42, 43]. Self-incompatibility (when encountered) can be overcome by culturing more seedlings in the same culture vessel[23].

Two serious impediments to seeding under *in vitro* conditions are a reduction in the number of florets maturing at the same time and a loss of synchrony in the timing of anthesis (exertion of androecium/gynoecium). Being a passive mode of pollination, in wind-pollinated plants it is essential that many flowers mature at the same time and anthesis (exertion of androecium/gynoecium) takes place nearly at the same time. In nature, flower opening is synchronized by a combination of three stimuli: a gradual increase in light intensity and atmospheric temperature, and a gradual decrease in relative humidity (as the sun rises). These stimuli are absent under *in vitro* conditions, where the

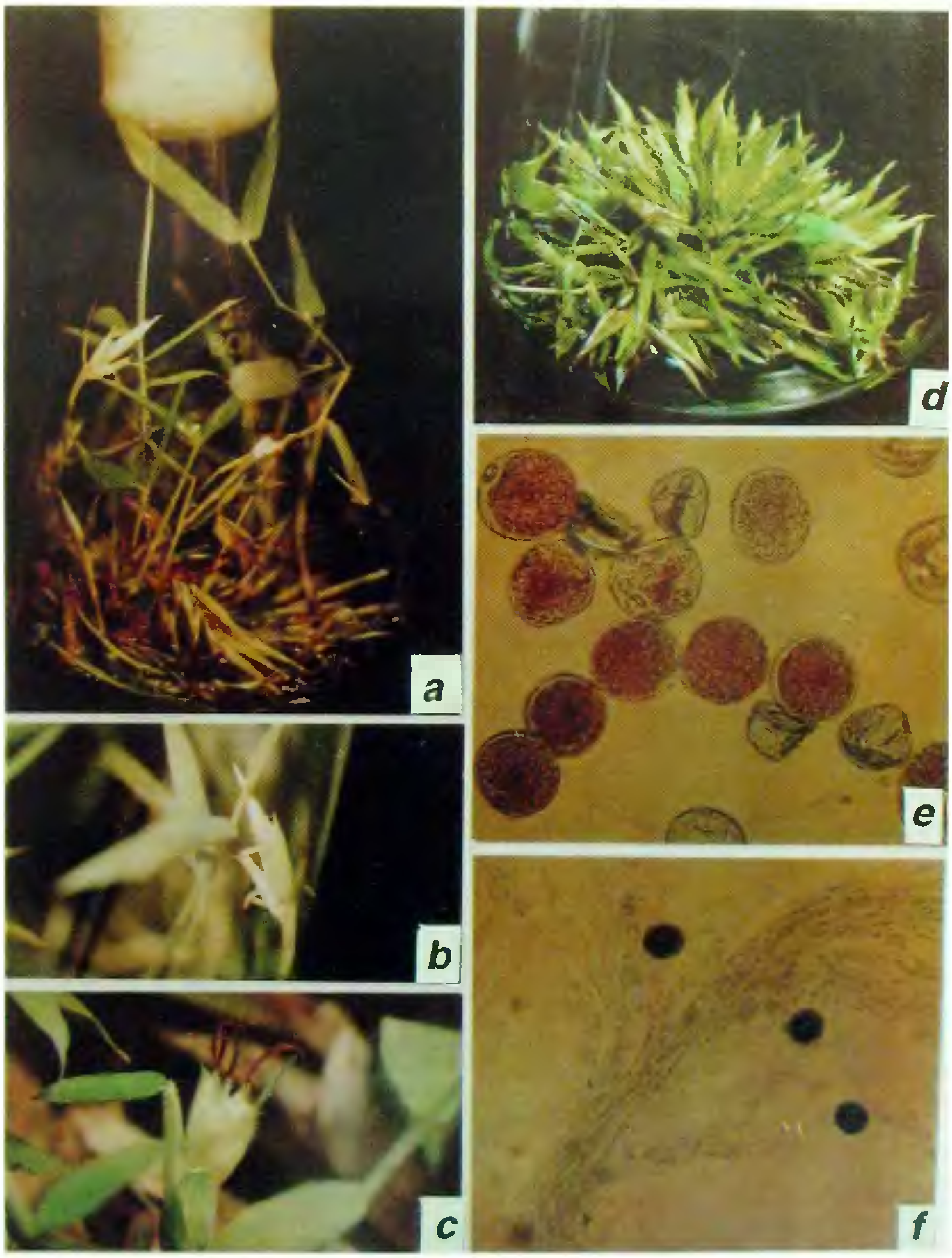

Figure 5. ***a***, *In vitro* induced flowering in *Bambusa arundinacea*; ***b***, close-up of a spikelet from ***a***, ***c***, one spikelet developed *in vitro* in *Dendrocalamus strictus*, ***d***, an inflorescence culture (***a–d***, courtesy Mrs Varsha Parasharami, NCL, Pune), ***e***, pollen from *in vitro* developed anthers of *Bambusa arundinacea* (stained with acetocarmine), ***f***, an enlarged view of a stigma from *in vitro* developed floret of *Bambusa arundinacea* (note pollen grains).

cultures are maintained in an ambient of constant light intensity and temperature, and humidity is always near saturation. Changes in light intensity are sudden (due to switching on/off of the fluorescent lamps). Exposing the cultures to daylight and room temperature conditions and reducing humidity in culture vessels are being tried. After successful fertilization, seed development may not pose serious problems. Wherever difficulties are encountered, embryo rescue may be helpful.

Conclusions

The importance of bamboos as a structural raw material to most of mankind cannot be overemphasized[1,4–6]. Throughout the long history of their use by Oriental civilizations, bamboos have never been improved by selective breeding[50], because of the long generation times characteristic of most of them. Methods for *in vitro* induction of flowering in bamboos, which otherwise takes many years to flower, has opened up endless possibilities[18–20]. It is necessary to look at the vast potential offered by bamboos, both for economic gains and for scientific exploration as a whole. There are many avenues open for increasing the production of bamboo raw material. Long-term storage of seeds, selection at seedling stage, somatic embryogenesis from mature explants, cryopreservation of pollen and rhizome transplantation from flowering clumps have special significance in bamboo propagation and improvement. Exploring these possibilities in bamboos should go side by side with efforts on applying modern technology for their improvement.

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