Bamboo Flowering & Tissue Culture

- Dilip Herlekar

Bamboo has a special place in human life. It is used for food, as a weapon, as a tool, as a cooking container, as a musical instrument as an object of beauty, as shelter or even as medicine. To the Asian, it provides him food and the wood for the home he lives in, the mat he sleeps on, the chopsticks he eats with, and the cup he drinks from. It provides a hat to shade his eyes from the sun and an umbrella to shelter him from the rain. He irrigates his paddy fields with a length of bamboo piping. He hangs his rice over a bamboo pole to dry and then sifts it through a bamboo sieve and carries it home in a bamboo basket. Then he has a good meal of rice and tender bamboo shoots and finishes his day with some music from a bamboo flute.

Bamboos are the most important items of forest produce used by the rural communities in Asia and the Pacific. It is reported that over 75 genera and 1250 species of bamboos occur in the world with the majority in the tropics. About 300 species are so far reported from China, 130 species from India, 55 species from Philippines, 50 species from Thailand, 33 species from Bangladesh, 31 species from Indonesia, 26 species from Papua New Guinea and 12 species from Malaysia. The largest forest area under bamboos is in India with 9.57 million hectares of bamboo forests or 12.8% of the total forest area. The principal bamboos are *Dendrucalatnus strictus* and *Bambusa arundinacea* with an overall annual production of 5 million tons. In China 3.4 million hectares are under bamboos (3% of total forest area), again with a production of 5 million tons. Two thirds of the area is under *Phyllostachyas pubescens*.

Scarcity of Bamboo Resources :

One of the most important contributions of bamboo to modern-day man is in the production of paper. Though once called poor manȏs timber, it is no longer cheap. Its use as a long-fibre raw material in the pulp and paper industry is well known and it is one of the most sought after raw materials in the tropics. Its use for making paper, however, dates back to more than 1,700 years ago. Such bamboo-made paper became world famous during the Tang Dynasty. "There are bamboo tiles for shelter, bamboo hats for shading, bamboo paper for writing, bamboo rafts for carrying, bamboo skin for clothing, bamboo shoes for wearing, bamboo shoots for eating and bamboo fuel for fires. Indeed we cannot live without bamboos for a single day." said Su Dongpo, the famous poet of the Song Dynasty in China,

In India, industrial production of paper was started in the 1930s. Since independence, however, there has been a rapid expansion of paper factories, with 35 of them using bamboo as a source of long-fibre After the Second World War, Sri Lanka and Indonesia also set up paper and pulp factories. China recently set up several small-scale paper factories using bamboo pulp.

At one time the supply of bamboo was thought to be perpetual. Bamboo was often viewed by foresters, in many countries, as a weed species and a nuisance due to its rapid growth, and therefore its mass-utilization in the paper industries was welcome. This impression soon proved to be a mirage. The rapidly increasing population with associated demands for fuel and farmland, resulted in a significant decrease of land area under forest in Asia (including that under bamboo). Between 1960-80, one-half of the increase in food or plantation production in Southeast Asia was achieved by extending plantation areas under cultivation by clearing forest land. In addition to the decrease in forest cover, overexploitation, bad management, lack of adequate state control over natural forest stands and vested interests have resulted in a situation where the forest stock of bamboo and the total annual net growth has decreased and will continue to decrease significantly in Asia.

The shortage of supply has been compounded by absence of or inadequate replantation. Also flowering (bamboo being monocarpic) results in the death of the entire clump. Populations of a given bamboo species belonging to the same provenance have been known to flower simultaneously irrespective of their planting locations. Seeds of *Thyrsostachys oliveri* that flowered in Burma (in 1891) were planted in Calcutta and at Dehra Dun which are 1500 km apart. Yet, at both places, the clumps flowered synchronously in 1940. The synchronous flowering of *Melocarlna baccifera* was observed in Garo Hills (Assam) and Dehra Dun. Thus the period between gregarious flowering of a species over the same area seems to be constant and cyclic. Such a phenomenon has also been recorded in other countries. In many states in India, assessments of bamboo wealth have been upset by subsequent gregarious flowering and the death of bamboo clumps following seeding. The resultant regeneration takes time to establish and has to face factors such as intense grazing pressure and forest fires.

The mass-utilization of bamboo by the paper and pulp industry that is quickly taking the plant out of reach of the common man. Its prices have shoot up and therefore Bamboo is no longer remains the poor manôs timber. On the other hand, it is fast becoming a high-value crop. In certain countries growing bamboos for shoots has proved to be more profitable than rice cultivation. These facts warrant reconsideration of the classification of bamboos as a óminor forest produceô in some countries and in others as ónon-commercial speciesô. This in itself would serve to bring a new focus on the bamboos and help in their conservation and replantation. As of now, many areas in countries where bamboos grew in dense thickets a decade ago now lie barren, save for a few isolated clumps, or the land has been reserved for other purposes after clearing bamboos.

Replanting Bamboo:

There is a growing realization that to combat the rapidly dwindling natural resource, large-scale replanting needs to be done. Replanting efforts using offsets or culm cuttings are slow and expensive. Only propagation with seeds is cheaper and easier with the seedlings being raised in nurseries and transplanted to the forest. This, however, offers only a limited answer to the problem since most of the larger, economic bamboos flower only once every 30-60 years. The sporadic flowering that takes place annually in isolated clumps yields, few viable seeds from a large mass of empty florets. Viable seeds obtained from gregarious flowering also suffer much damage due to rodents, insect attack and rapid loss of viability due to poor storage. Clearly, research into newer and more rapid methods of propagation is urgently called for.

Bamboo Mass-Propagation Through Tissue Culture:

Plant tissue culture offers the unique opportunity not only for realizing the totipotency of cells into whole plants but also for providing conditions under which physiological manipulations can be carried out with the objective of overcoming endogenous controls inherent in the intact plant. Micropropagation through nodal segments and somatic embryogenesis are methods which could help the regeneration of large numbers of plants in a relatively short time.

At the Department of Botany, University of Delhi, there is active research in progress using both the above mentioned methods for mass producing bamboo plantlets. By using embryogenic cultures and the embryoids resulting therefrom, any number of plantlets can be produced. The application of the method of micropropagation is, however, still limited, since there has been partial success using nodes or other materials from mature, adult bamboo plants by which ócloningô of these (making multiple, identical copies of the parent plant) could be achieved. So far only about 4-10% of the shoots obtained from mature nodes (depending on the species) in culture can be induced to root. Better success has been obtained with seedling material but this does not satisfy the objective of cloning mature, elite bamboos.

In comparison, using the method of somatic embryogenesis, large-scale propagation of bamboos is possible at minimal cost and with the lowest labour input. Whereas this is also possible at present from juvenile tissues, recent research in the laboratory at the University of Delhi is showing that the barrier posed by mature tissues may be eliminated. The work described below out-lines the method followed to mass-produce bamboos in tissue culture through somatic embryogenesis and the successful planting of these tissue-culture raised bamboos in several forest areas of India.

In this laboratory, somatic embryogenesis, has been achieved in three bamboos, namely, *Dendrocalamus strictus. Bambusa arundinacea* and *Bambusa balcoa.* Embryogenic cultures can be initiated from a wide range of juvenile explants. Immature embryos, mature embryos, and mesocotyl, node, leaf sheath, leaf and root of the young seedling can be used. Newly initiated rhizomes can also be utilized for raising embryogenic cultures. Whereas seeds are commonly sterilized with a chlorine-releasing agent or with chlorine water, explants taken from the field are sterilized with mercuric chloride. Seedling explants are ordinarily taken from in vitro raised seedlings.

After dehusking, the mature seeds are washed in 2% Teepol solution on a magnetic stirrer for 5 min. The Teepol solution is removed by washing in running tap water for 15-20 min. followed by a rinse in distilled water. Sterilization of the material is effected by a 5-min immersion of seeds in chlorine water (saturated chlorine water diluted five times with distilled water) followed by thorough washing in sterile distilled water. Surface moisture is removed with a sterile filter paper and the seeds implanted in tubes containing the inductive medium under aseptic conditions.

The inductive medium consists of salts and vitamins of B5 basal medium. The pH of the medium is adjusted to 5.8 prior to autoclaving at 15 psi for 15 min. The cultures are maintained at 27 ± 2°C under continuous illumination (2,500 lux) provided by cool white daylight fluorescent tubes.

Callusing starts soon after germination of the zygotic embryo. Both friable and compact calli are formed. The compact callus was whitish to creamish in colour. Even when mature nodes are used, similar embryogenic compact callus is induced. Callus origin was traced to the vascular bundles. Cells giving rise to the embryogenic compact callus are first recognizable by the densely packed starch contents and thick walls. These cells become meristematic and initially undergo transverse divisions. Starch breakdown accompanies further cell divisions and continued divisions lead to compact callus formation. Sometimes, the cells adjoining the bundles are also induced. The somatic embryos arise as protuberances on the compact callus. Subsequently, two humps form on top and the tissue at the back grows up into a green hood-like scutellum. The enclosed central region forms the shoot pole whereas the root pole differentiates below it. The general appearance of the callus, at this point of time, varies from white to cream with green areas where the embryoids are maturing and becoming chlorophyllous.

In comparison to multiplication of embryogenic compact callus followed by differentiation of embryoids, secondary somatic embryogenesis has been found to be more rapid. In this process the primary embryoids proliferate further and give rise to a new crop of secondary embryoids. These arise by the proliferation of the scutellar epidermis. Numerous embryoids develop further from the proliferated epidermis. A further crop of embryoids can be obtained from these secondary embryoids and the process continued indefinitely until removed from the permissive medium.

If left on the initial inductive medium, the mature embryoids germinate and produce both shoot and root. The plantlets thus produced can be separated and transferred to pots after an in vitro growth period of 1 month. At present, the method followed is that the surplus embryoids from a round of embryoid multiplication are transferred for maturation and germination. Here the embryoids are given an equal opportunity to mature and germinate into plantlets. The plantlets formed are removed to a medium that permits them to grow further or precocious rhizome induction is carried out. The un-germinated embryoids are then re-transferred for another round of embryoid maturation and germination. About 30% of the mature embryoids germinate in one round of subculture. At the present stage it has not been possible to evolve a protocol that permits simultaneous maturation of all embryoids on semisolid medium and their subsequent simultaneous germination.

Potting of Plantlets and Acclimatization :

The plantlets are potted out directly after removing from the medium. These are attained in the acclimatization chamber for 2- 3 weeks and then removed to the greenhouse for further growth The plantlets are subsequently transferred to polyethylene bags, maintained in the open and handed over to forestry agencies when they are about 4-12 months old. Normally, planting in the forest is not done before 8 months and is generally carried out when the plants are 12 months old. The rhizome system is well developed by this time and the plants establish easily.

Acclimatization chambers available in market are costly. Hence, simple but useful chambers have been developed by Delhi University. Mass-production of tissue-culture raised plantlets would necessarily require a large facility for acclimatization of the plantlets. A facility of two chambers has been designed and set up in Delhi University laboratory for keeping the plantlets under lighted conditions. Up to 12,000 plantlets can be acclimatized at a time. Considering that bamboo plantlets need hardening for 3 weeks (for *Dendrocalamus strictus*; 2 weeks for *Bambusa arundinacea*), around 0.2 million plantlets can be acclimatized in both chambers in one yearȇs time.

Packaging & Transport :

Initially the hardened plantlets used to be potted into large 25-cm diameter earthenware pots and transported as such. This has now been changed to 15-cm diameter polyethylene bags with holes in them, although our experience has shown that the plantlets perform much better in 25-cm diameter polyethylene bags. These changes have been made in deference to the requirements of the foresters as also keeping in mind the easier transport with polyethylene bags. Alternatively, the plantlets are removed from the bags, the earth balled up around the roots, and the plantlets put together and tied in bundles of 25 each. This bundle is then wrapped in a layer of moss and newspaper, and put into a large polyethylene bag. This method is suitable for air-transporting plants and is relatively inexpensive as 500 plants required for planting 1 hectare weigh only 50 kg and can be transported easily in cardboard cartons. However, such plants require replanting and care in a nursery before planting in the forest.

Cost:

At present, the cost of production of mature bamboo plantlet is **Rs 5.00,** and **Rs 2.50** to acclimatize and rear in the field for 8 months. The efforts are on to reduce this cost with improvement in procedure and reduction in cost culture.

Conclusion:

Thus, short supply of seeds, due to number of reasons in case of Bamboo has been overcome with tissue culture method to great extent, at reasonable cost and good success rate. It needs to be remembered that, conventionally, one seed ordinarily gives rise to only one plant whereas numerous plantlets can be through the method of somatic embryogenesis. By this method, one seed can "plant" a whole and perhaps several hectares. If the plants prove to be of good quality, a whole hectare of monoclonal, superior plants will be available for further propagation by conventional methods. Besides, the original cultures can be maintained and can give rise to several more plants. Tissue culturing of bamboos would also lead to the isolation of superior somaclonal variants, induction of in vitro flowering and an understanding of rhizome physiology.

Above all, it will lead to the restoration of our bamboo forests to their earlier green glory and that is the best outcome of science and technology in this field for all of us.
