Chapter 1

Introduction- General
As the 21st century approaches, the world is facing problems which seem to be intractable. Irrigation water is in short supply in many parts of the globe and erosion threatens the productivity of much farmland. While the amount of land under cultivation cannot be expanded greatly, almost 100 million people are expected to be added to the world's population each year for the next 30 years. It has been estimated that by the year 2020, the global population may reach a level of eight billion. The proportion of such population living in developing countries will be 80% approximately. As a result, per capita food production is likely to continue to decline in sub-Saharan Africa and barely rise in South Asia (Hileman 1995). Globally, annual food production will have to go up to about 3000 million metric tonnes from the current 1800 million metric tonnes. Even now the per capita arable land availability is only 0.1 ha in China and 0.15 ha in India. There is thus no option except to produce more from less land to meet the needs of growing population.

Agriculture is the main occupation of the people living in most countries, especially the developing ones. In the agricultural sector, it is absolutely essential that the production and productivity of the major food and economic crops be pushed up, in the face of growing world population. Driven by the need to grow more food from less land, scientists have been trying out all sorts of combinations and permutations of desirable nature to augment crop improvement. The yields of major crops (cereals) have now reached a plateau, the conventional techniques have been well exploited, and any further yield gains are unlikely without using newer approaches (Ignacimuthu 1996).

**Biotechnology at the rescue of Agriculture**

The advent of biotechnology has opened up new opportunities for plant improvement by rendering novel genetic combinations feasible. Biotechnology is the key technology for future developments. Plant biotechnology is the application of current scientific methods and techniques to agriculture, horticulture, forestry, energy, food, pharmaceutical, cosmetic and service industries. Agricultural biotechnology has progressed to a stage wherein specific characteristics to improve the yield, appearance, disease resistance, nutritional quality and adaptation to adverse soil conditions can be built into the targeted crop. In recent years plant biotechnology has also contributed to the broadening of genetic base of the existing varieties of crop plants.

Use of totipotency of plant cells and formation of many shoots from organised structures -meristems- has made mass propagation of species possible, for which there is no other adequate way of producing large numbers of plants in a short time. The ability to manipulate plant cells,
tissues and genomes in vitro by the technique of plant tissue culture has opened up many possibilities such as haploid production, hybrid production through embryo rescue, mass clonal propagation of elite breeding materials, mutant selection, somatic cell hybridization and genetic transformation. Recombinant DNA technology has resulted in identification and isolation of a large number of useful genes from various taxa including microbes. These techniques are now being used successfully to produce important plants with improved agronomic traits by mobilising genes of interest from related or unrelated species or genera. Biotechnology will represent 10-15% market share of several traditional agribusiness sectors like seeds and pesticides, with considerable sales coming from new markets such as pharmaceuticals manufactured in transgenic plants and animals. The major route for the application of new technology in crop agriculture will be through the development of new plant varieties with novel traits.

Plant biotechnology in India has to provide not only more food but also sustained employment and income generation. We already have about 50% of land area under cultivation. There is thus greater need for concerted efforts to ensure food security at the household level in the years to come. India has an agri-based economy and the role of new technologies in agriculture and industry has more relevance for the country than in other areas. The abundance of genetic bases, diverse agro-climatic zones and highly qualified manpower offers rich scope for technological advances in agricultural and industrial biotechnology. India has been a leading exporter of primary produce such as tea, coffee, rubber, cardamom, cotton and various other species including aromatic and medicinal plants. Of late, India's premier position has been surrendered to other developing countries such as Guatemala, Kenya and Brazil due to noncompetitive prices and low productivity. Thus the need for use of the tools of plant biotechnology particularly tissue culture and related techniques in increasing the productivity is felt more today than at any time in our history.

Importance of Tissue Culture in Biotechnology

An important aspect of all biotechnology processes is the culture of either microorganisms or plant and animal cells (or protoplasts in case of plants) or tissues and organs in artificial media. Plant cell and tissue cultures are used for a variety of genetic manipulations as with microbial culture systems. For example anther culture is used for haploid breeding, gametic and somatic cell/ tissue cultures are used for tapping gametoclonal and somaclonal variation or for production of artificial seeds. Transformation of protoplasts in culture leads to the production of useful transgenic plants. However, complete in vitro regeneration protocols of the transformed plant cell/tissue is one of the important prerequisites for producing transgenic plants by any means of transformation (vector mediated like Agrobacterium mediated or any other physical methods like particle bombardment). Embryo culture technique has also helped in extending the range of distant hybridisations for plant breeding purposes (Gupta 1995).
Plant cell and tissue culture generated much excitement during the last decades concerning the potential application of the technology for improving important agricultural crop plants. There are several areas of *in vitro* culture which have potential practical application. However, currently, the most important practical application of these techniques is for cloning or mass multiplication of selected genotypes. This is evidenced by the large number of commercial firms engaged in propagating a variety of plants through tissue culture.

**The need for tissue culture of fruit and forest tree species**

Trees have comparatively long generation cycles, so breeding and conventional improvement programmes are tedious and take a long duration compared to that of annual crop species. Also these programmes are long term and require large areas of land for testing of selected genotypes and for production of seedlings for plantations or afforestation programmes. In many of the tree species there is very low fruit setting because of heavy flower drop, fungal susceptibility, failure of pollination or some physiological mechanism. As many of the tree species are cross pollinated, selfing followed by selection is not easily possible. The heavy deforestation resulted in loss of genetic diversity of many of the forest and fruit trees. Even if some elite tree species is identified, conventional seed propagation cannot be relied upon for multiplication of these trees because

- the seedling trees are slow growing
- late in bearing flowers and fruits
- the cross pollination in most of the trees species will results in loss of unique characteristics
- the occurrence of wide variability among the seedling populations.

Conventional propagation methods through vegetative means are not always successful and many a time cause the destruction of the mother plant. In this context, improvement of forest and fruit species using *in vitro* techniques is more relevant. Although tissue culture will not supplant traditional tree improvement efforts, the researchers may be able to use these techniques to evaluate genotypes for better growth rate, cold hardiness, disease resistance and tolerance to drought or to chemicals such as alkaline/acidic soil, salts or herbicides which would have taken much longer and would have required many hectares of land to accomplish (Karnosky 1981).

Haploid plants can be produced through anther culture, which can be utilized for breeding programmes or to induce gametoclonal variations. Indirect regeneration protocols through callus and differentiated structures can be used for alien gene transformation, and introduction of new traits and desirable qualities in species which does not have such genes or can be explored for somaclonal variation and thus the genetic base of the these species can be increased in short duration. Protoplast isolation, culture and fusion techniques can be utilised in transferring genes from related or unrelated species which are sexually incompatible. Somatic embryogenesis and
synthetic seed technology as well as micropropagation protocols can be utilised for multiplication of identified elite as well as endangered trees for cultivation and for large scale social forestry plantations.

**Brief historic perspectives of plant tissue culture**

The first attempt to keep isolated organs alive dates back to the beginning of this century. The first real step in *in vitro* culture were taken by a German botanist Haberlandt (1902). He kept alive little clusters of cells (stamen hairs, glandular hairs or epidermal peels) for several months in ameliorated Knop's medium. But there was no cellular multiplication and the experiments to induce growth were ultimately a failure.

In 1922 Robbins of USA and Kotte of Germany working on root tips (a few mm long) succeeded in keeping them alive for nearly six months and obtained fragments that grew 5 - 6 cm in length. The fragments later stopped growing and eventually showed senescence.

White (1934) obtained for the first time an indefinite culture of roots of tomato in liquid medium containing mineral salts, yeast extract and sugar. In 1934 Gautheret could achieve proliferation of tissue from isolated cambium, which unfortunately did not survive longer than eight months. It was in 1939 that Gautheret published his first results on the indefinite cultures of carrot tissues. Nobecourt (1939), working with the same material and White (1939) on tumorous tissue of tobacco published analogous results in the same year. *In vitro* culture of plant tissue really began from that time.

Another historic stage was the recovery of disease free plants stock infested with virus with the help of *in vitro* meristem culture. In 1949 Limasset & Cornuet published their observations on the absence of virus in the meristem of viral infected tobacco plant. Morel & Martin (1952) profited by these observations and generated *in vitro* cultures of meristem isolated from dahlias and potato plants damaged by viral diseases. From these meristems they obtained entire plants *in vitro* in which symptoms of disease subsided in normal culture and eventually became healthy. Thus the technique of micropropagation was available in 1952 which is now universally used to produce plants free of all sorts of virus infections as well as for mass multiplication (Gautheret 1985, Beauchesne 1995).

**Tissue culture in India**

Research on plant cell and tissue culture were initiated as early as 1960's at Botany Department, Delhi University, with important contributions in the areas of haploid production and anther culture methodology. There after many protocols were standardised for a number of plant species in various laboratories. India is now reported to have one of the largest groups of tissue culture
scientists in the world. Mostly the research is directed towards the development of improved plants for agriculture, horticulture and forestry using tissue culture methods. Another use of tissue culture methods in some laboratories involve studies in the areas of developmental biology, biochemistry, physiology, genetics and molecular biology to answer the questions of fundamental nature.

The department of Biotechnology (DBT), New Delhi is playing a special role in promoting research in the area of plant tissue culture by financially supporting many of the national laboratories and University research programmes through sponsored projects. Recently, DBT has sponsored a project on selection on panama disease resistant banana plants through somaclonal variation approach in this laboratory. Besides these laboratories, several private companies are also engaged in commercial production of many plant species (Gupta 1995).

Commercial tissue culture in India

Advancements in commercialisation of plant tissue culture and acceptance of tissue cultured plantlets by the agricultural sector have led to the continued exponential growth within the industry in terms of number of new units as well as number of plants produced by these units. Although Indian scientists are credited for reporting several important breakthroughs in the area of plant tissue culture, unfortunately, very few commercial units in India have been set up with indigenous know how. Most of our commercial units prefer to seek the entire technology package on a turn key basis and, in fact have been set up in foreign collaboration with M/S Curtiss and M/S Green Tek- Holland, Microplants Ltd.-UK, Centre de Recherches Agronomiques-Belgium, Plantex - Australia and Agrobio - France etc. with buy back arrangements for the initial period.

The earliest commercial micropropagation laboratory in India was set up in Cochin export processing zone (in 1987) under the name A.V. Thomas and Co. for the cloning of Cardamom plants from its own farm collections, using the technology provided by NCL Pune. Since then there has been an exponential expansion in Indian tissue culture industry from 4 units in 1988 to 75 in 1996, as well as their production capacity increasing from 5 million plants per annum in 1988 to about 190 million plants in 1996. Other main companies which followed the successful attempt of AVT are Indo-American hybrid seeds - Bangalore, Hindustan Lever Ltd - Mumbai and Unicorn Biotek - Hyderabad in 1988. Later many other companies came upto the market for example Cadila Ahmedabad, Parrys-Chennai, Godrej Biotech-Hyderabad, Kumar Genetics-Pune, Kothari Biotech-Bangalore etc.

An analysis of plants micropropagated by Indian industry shows that ornamental plants (Foliage and flowering plants) are the major items being produced in line with the international trend. Banana is the single fruit plant which is widely micropropagated by various industries all
throughout the country. In addition to the ornamentals, Indian units are currently producing fruit crops, forest trees, vegetable crops and plantation crops (Govil & Gupta 1997).

**Importance of the plants selected for the present study**

*Emblica officinalis*

Aonla or Amla (*Emblica officinalis* Gaertn.) belonging to the family Euphorbiaceae, known also as Indian gooseberry is one of the very important minor fruit crop of commercial significance of the subtropical and tropical climate (Fig. 1a-b). It is native of India, Malaya and China. It is a quite hardy tree, prolific bearer and highly remunerative even without much care (Bajpai & Shukla 1990). Aonla has vast scope of growing in wastelands, salt affected soils and marginal lands. So it is a highly preferred tree for social forestry-afforestation programmes (Mathur & Bordia 1994).

It is a small or medium sized deciduous tree with smooth greenish grey, exfoliating bark. The shoot system has two kinds of branches, one is the normal branches of indefinite growth and the other is branchlets of limited growth on which the simple leaves are arranged alternatively and the whole structure resembles a pinnately compound leaf. Fruits are depressed globose, 1-3 cm in diameter, fleshy and obscurely 6 lobed containing 6 trigonous seeds. The tree is common in the moist deciduous forests of India, ascending to 4500 ft on the hills. It is also cultivated in orchards and in home yards.

There are many cultivars of Aonla like, Banarasi, Chakkaiya, Francis, Anand I and II etc. There are also some new selection with good agronomic characters like low fibre content, higher yields, high vitamin C content etc. and some of them are given in Table-1.1.

The yield of Aonla fruit varies with variety, soil conditions, maintenance of plantation etc. However eight year old plants of Aonla - Kanchan, Amrit, and Neelum- grown in wastelands (salt affected soils) produce 8-11 tonnes of fruits giving a net profit of Rs- 14,000/- to 21,000/- per hectare (Singh et al 1994).

*Emblica officinalis* is usually propagated by seeds. It may also be propagated vegetatively through budding, cutting, grafting and inarching. The plant is sensitive to frost and drought. It coppices well and pollards moderately well. The coppice shoots in particular grow vigorously. The flowers usually appears in the hot season and fruit ripens during the following winter. Aonla occurs in the first week of March. The flowers commence opening from the last week of March and the blooming period lasts for 3 weeks. Male flowers appears in clusters in the axil of leaf all
Fig. 1  

a A three year old Aonla tree (*Emblica officinalis* cv NA7) in fruiting at the Horticultural Research Station, Gujarat Agricultural University, Anand

b An 18 year old Aonla tree (*E. officinalis* cv Anand-2) in fruiting at the Horticultural Research Station, Gujarat Agricultural University, Anand
over the branchlet while female flowers on the upper end of a few branchlets only. The sex ratio (male to female flower) is 307.9:1 to 197:1 depending on the variety and environmental conditions.

Table-1.1: Agronomic characters of some cultivars of Aonla

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Size</th>
<th>Pulp %</th>
<th>Fibre %</th>
<th>Total soluble solids %</th>
<th>Acidity %</th>
<th>Ascorbate (mg/100 gm)</th>
<th>Yield/8 year old plant (kg)</th>
<th>Season of harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanchan</td>
<td>medium</td>
<td>90</td>
<td>2.5</td>
<td>11.3</td>
<td>2.2</td>
<td>711</td>
<td>74</td>
<td>Nov.</td>
</tr>
<tr>
<td>Krishna</td>
<td>large</td>
<td>93</td>
<td>14</td>
<td>14</td>
<td>2</td>
<td>783</td>
<td>41</td>
<td>Oct.</td>
</tr>
<tr>
<td>Amrit</td>
<td>medium/large</td>
<td>94</td>
<td>0.8</td>
<td>11</td>
<td>1.7</td>
<td>707</td>
<td>56</td>
<td>Dec.</td>
</tr>
<tr>
<td>Neelam (Fig. 1a)</td>
<td>medium/large</td>
<td>93</td>
<td>1.5</td>
<td>10.7</td>
<td>1.9</td>
<td>788</td>
<td>62</td>
<td>Dec.-Jan.</td>
</tr>
<tr>
<td>Anand II (Fig. 1b)</td>
<td>medium</td>
<td>91</td>
<td>1.8</td>
<td>7.55</td>
<td>1.39</td>
<td>780</td>
<td>65</td>
<td>Dec.-Feb.</td>
</tr>
</tbody>
</table>

Aonla is wind pollinated as the pollen grains are light and are produced in abundance. There is no self incompatibility in Aonla and the cause of poor fruit set (12-18%) may be due to a high percentage of staminate flowers. An increase in fruit set with hand pollination (18-27%) indicates that need of pollinating agents for better setting (Bajpai & Shukla 1990).

Flower and fruit drop occurs at three stages. The first drop is the highest as 70% of the flowers drop off within three weeks of flowering due to degeneration of egg apparatus and lack of pollination. The second drop occurs from June to September due to lack of pollination and fertilization. The third drop consists of fruits of various stages beginning from third week of August until October probably due to embryological or physiological factors.

After the fruits have set, the embryo lies in dormant condition and ovary does not exhibit any symptoms of external growth until middle of August. The diameter and volume of the fruit increases rapidly thereafter and maximum growth is achieved by November after which there is not much increase in size. The growth of the fruit is due to the enlargement of the mesocarp while endocarp cells form the hard stone cells. The reason for the long dormancy of the fruits after fertilization before development is unknown.
The fruit is green when tender, changing to light yellow or brick red colour when mature. It is sour and astringent and is occasionally eaten raw. It is much esteemed for making pickles, preserves and jellies. The fruit is a rich source of pectin. Aonla fruit is the richest source of natural vitamin (among fruits except Barbados cherry). The fruit juice contains nearly 20 times as much vitamin C as orange juice and a single fruit is equal in antiscorbutic value to 1-2 oranges. A tannin containing gallic acid, ellagic acid and glucose in its molecule and naturally present in the fruit, prevents or retards the oxidation of vitamins and renders the fruit a valuable antiscorbutic in fresh as well as in the dry condition. The antiscorbutic value is well conserved by preserving the fruits in salt solution or in the form of dry powder. The dried fruits losses only 20% of its vitamins in 375 days when kept in refrigerator but losses 67% in the same period when stored at room temperature.

Feeding trials on healthy human subjects suffering from pulmonary tuberculosis, vitamin C saturation is more quickly reached with Aonla powder than with synthetic vitamin C, thereby showing that the former is readily assimilated probably due to the presence of accessory factors. The Aonla fruits were also used successfully in the treatment of human scurvy (Anonymous 1952).

Aonla fruit has been held in high esteem indigenous medicine. It is acrid, cooling, refrigerant, diuretic and laxative. The raw fruit is eaten as an aperient. Dried fruit is useful in haemorrhage, diarrhoea and dysentery. In combination with iron, it is used as a remedy for anaemia, jaundice and dyspepsia. A fermented liquor prepared from the fruit is used in jaundice and cough. Acute bacillary dysentery may be arrested by drinking a sherbet of Aonla with lemon juice. Triphala consisting of equal parts of powdered emblic myrobalan (Emblica officinalis), chebulic myrobalan (Terminalia chebula) and belleric myrobalan (Terminalia bellirica) is used as laxative and in headache, biliousness, dyspepsia, constipation, piles, enlarged liver, and ascites. The exudation from incisions on the fruit is used as an external application for inflammation of eye. The flowers are also cooling, refrigerant and aperient as the fruits. The root and bark are astringent (Anonymous 1952).

The fruit pulp is used against a variety of disease conditions such as liver injury and atherosclerosis (De et al 1993, Thakur et al 1988). Chyvanaprasc, a health tonic (the major content is Aonla) has been indicated as a rejuvenating preparation and recent studies have shown its action as an oxygen (free radical) scavenger. Studies have also revealed that both chyvanaprasc as well as extract of E. officinalis could scavenge superoxide and hydroxyl radicals and could inhibit lipid peroxidation (Jose & Kuttan 1995).
Recent studies carried out by Jose et al (1997) indicated the use of *E. officinalis* and chyavanaprasha as an anticarcinogen in 20-methylcholanthrene induced sarcoma formation in test animals and as an antimutagen against direct mutagens as well as mutagens that need metabolic activation. These workers have also reported that *E. officinalis* could inhibit the activity of Phase I enzymes which are needed for the activation of carcinogen and higher concentrations of *E. officinalis* could induce glutathione-S-transferase, a key enzyme in detoxification. Hence, the biological activity of *E. officinalis* has been attributed to a combined action of the following (a) removal of free radicals, (b) inhibition of phase I enzymes and (c) stimulation of phase II enzymes (Jose et al 1997). Moreover it has been shown to reduce serum aortic and hepatic cholesterol in animals fed with a high cholesterol diet (Thakur 1985). *E. officinalis* is also known to reduce the blood sugar in patients with diabetes (Tripathi et al 1979). It is additionally antimutagenic as tested in *Salmonella typhimurium* assays against polycyclic aromatic hydrocarbons (Grover & Kaur 1989). The extract has also shown to reduce the toxicity and clastogenicity induced by nickel, lead, aluminium etc. (Dhir et al 1991).

Components present in Aonla fruits include ascorbic acid (Mitra & Ghose 1942), tannins (Brahmachari & Gupta 1958), trigalloyl glucose (Damodaran & Nair 1936), flavanoids (Khanna et al 1982). Ascorbic acid present in plants is associated with polyphenols (Brahmachari & Gupta 1958) and its reducing activity was shown to be heat stable. Polyphenols present in fruits are ellagic acid (Damodaran & Nair 1936) and Phyllemblic acid (Pillay & Iyer 1988). But a comparison of the biological activity of the extract with its known components indicates that the extract may contain some unknown compounds with stronger antioxidant activity (Jose & Kuttan 1995).

Recent studies by Ghosal et al (1996) with the help of modern analytical tools like HPLC, HPTLC, Mass spectroscopy and NMR could not locate L-ascorbic acid in the pericarp of Aonla fruits either in free or in masked form. Thus the persistent claim that aonla fruit owes its therapeutic activity to its rich vitamin C (L-ascorbic acid) content is untenable. The potent vitamin C like activity of aonla has been located in the low molecular wt (M.Wt <1000) hydrolysable tannins. Four such compounds have been isolated and their structures established by conventional methods. The two new compounds, Emblicanin A and B, have been found to provide protection against oxygen radical induced haemolysis of rat peripheral blood erythrocytes, in a dose dependent manner.

In addition to the medicinal uses of the fruits, they are also used in the preparation of writing inks and hair dyes. The dried fruit is detergent and is used as shampoo for the head. An oil extracted from the fruit have the property of promoting hair growth (Anonymous 1952).
The seeds are used in the treatment of asthma, bronchitis and biliousness. They contain an oil, phosphatides and small quantity of essential oil with a characteristic odour. The fruits, barks and leaves are rich in tannin. The distribution of tannin in the plant is as follows. Fruits 28%, twig bark 21%, stem bark 8-9% and leaves 22%. Immature fruits are employed for tanning in combination with other tanstuffs such as Myrobalans. The twig bark is of considerable value as a tanning material.

The leaves and fruits are used as fodder for cattle. The leaves contain a brownish yellow colouring matter used in dyeing tussur and mulberry silks and wool. When used in iron mordant a black colour is produced. The leaves are used as manure in Areca and cardamom plantations. They may also be employed for ameliorating alkali soils.

The wood is red, hard and close grained. It is liable to split. It is used for agricultural implements, poles and furniture work. It is durable under water and is suited for well work. It is also used as fuel and for making charcoal.

**Previous in vitro studies on Aonla**
Although *Emblica officinalis* is a plant of high medicinal and economic importance, it did not catch much attention of researchers in the field of tissue culture. Till now there are only three reports on the tissue culture studies on this plant to our knowledge. Sehgal & Khurana (1985) have reported morphogenesis and plant regeneration from cultured endosperm of *E. officinalis*. They have noticed somatic embryo like structures in the callus produced from endosperm. But establishment of plantlets in the field was not achieved. Though they obtained shoots from callus rooting of shoots was not achieved. Recently Gupta et al (1994) and Verma & Kant (1996) have reported the multiplication of seedling explants of Aonla through callus cultures.

As a plant of high medicinal importance and because of recent findings of new antioxidant compounds in the fruits of Aonla, it has attracted attention of geneticists and biotechnologists for improvement of the plant through somatic hybridization, genetic engineering, *in vitro* mutagenesis, large scale micropropagation of elite identified plants or for secondary metabolite production. For all these programmes, reproducible and less cumbersome protocols for propagation under *in vitro* conditions are required.

*Achrassapota*

*Sapota*, belonging to the family sapotaceae, is an evergreen tree with spreading crown attaining a height of 20-30 ft (Fig. 2a). Flowers appear throughout the year and the fruits ripen mainly
Fig. 2  
a  A 18-20 year old tree of Chikoo (*Achras sapota* cv Kalipatti) growing at the Central Fruit Nursery, Gujarat Agricultural University, Vadodara

b  An 80-100 year old tree of Khiri (*Manilkara hexandra*) growing at the Botanical garden, M.S. University of Baroda
during March-April and August-September. Sapota is a delicious fruit introduced from tropical America. It is also known as sapodilla or Chiku. In India, it is mainly cultivated for its fruits, while in Mexico, Guatemala, British Honduras and other countries chicle gum is commercially produced. Immature fruits are astringent, while ripe fruits are sweet smelling and delicious. The mature fruits are also used for making mixed jams and they provide a valuable source of raw material for the manufacture of industrial glucose, pectin and natural fruit jellies. They are also canned as slices. In Dutch East Indies, the young leafy shoots are frequently eaten raw or mixed with other vegetables like lab-lab and consumed as vegetable after steaming.

Sapota when fully ripe is delicious and is eaten as dessert fruit (Table-1.2). The pulp is sweet and melting. The usual practice is to eat only the pulp. The fruit skin can also be eaten since it is richer than the pulp in nutritive value. The sapota fruits are good source of sugar which ranges between 12 and 14 percent. The pulp is also made into sherbet and halwa.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Amount (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>73.7</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>21.4</td>
</tr>
<tr>
<td>Protein</td>
<td>0.7</td>
</tr>
<tr>
<td>Fat</td>
<td>1.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.028</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.027</td>
</tr>
<tr>
<td>Iron</td>
<td>0.002</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The unripe fruits and bark contain latex much (20-25%) of which consists of a gutta percha like substance (chicle gum). The trees can be tapped once in three years and yields about 6-8 lb of gum. Frequent tapping devitalises the plant and consequently results in low bearing of fruits. It is used for making chewing gum. Other uses of chicle gums are in dental surgery, as a substitute for gutta percha and for making transmission belts. The bark also contains 11.8% tannin and is used by fishermen in South Luzon for colouring ships, sails and fishing tackle. The seed kernel contain 20% liquid fat, 1% saponin and 0.08% of a bitter principle sapotinin. The timber is also said to be very durable. In the coastal areas, the fruits are soaked in melted butter for a night and eaten in the morning. It is said to be an excellent preventive against biliousness and febrile attacks. In the West Indies, the seeds are known to be aperient and diuretic and the bark is reputed to be

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tonic and febrifuge. In Guinea, the bark is used as a tonic and antipyretic. In Kampuchea, the bark is considered as an astringent and febrifuge. The decoction is given in diarrhoea and paludism.

Sapota is a native of Mexico and Central America and now widely cultivated throughout tropics. The states that are growing sapota on a commercial scale in India are Maharashtra, Gujarat, Andhra Pradesh, Karnataka, West Bengal, Punjab, Tamilnadu, Kerala, Uttar Pradesh and Haryana. The total area under sapota cultivation in India is about 5000 hectares.

Table-1.3: Some cultivars of A. sapota grown in India

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>States</th>
<th>Bearing</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaiipatti</td>
<td>MH, GJ, KA</td>
<td>heavy bearer</td>
<td>oval, less seeded, sweet mellow flesh of excellent quality</td>
</tr>
<tr>
<td>Chaatri</td>
<td>MH</td>
<td>&quot;</td>
<td>quality not good as Kaiipatti</td>
</tr>
<tr>
<td>Long</td>
<td>MH</td>
<td>poor bearer</td>
<td>long, thin, very sweet</td>
</tr>
<tr>
<td>Bhuri</td>
<td>-</td>
<td>medium</td>
<td>good quality large fruits</td>
</tr>
<tr>
<td>Pala</td>
<td>AP, TN</td>
<td>heavy, in clusters</td>
<td>small to medium sized and oval or egg shaped, very sweet, good flavour</td>
</tr>
<tr>
<td>Kirthabharthi</td>
<td>AP</td>
<td>medium</td>
<td>Small to medium sized, oval, 3-4 ridges, sweet</td>
</tr>
<tr>
<td>Jonnavalasa</td>
<td>AP</td>
<td>medium</td>
<td>small to medium size, round 10-11 ridges. The pulp is firm and cream coloured and sweet</td>
</tr>
<tr>
<td>Cricket ball /</td>
<td>TN, KA, MH, WB, AP</td>
<td>shy bearer</td>
<td>largest sized fruits, round, pulp is gritty and granular and not very sweet</td>
</tr>
<tr>
<td>Calcutta large</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangalore</td>
<td>AP</td>
<td>medium</td>
<td>large, oval fruits with nine ridges, pulp gold coloured with tinge of musk melon, medium sweet</td>
</tr>
<tr>
<td>Jonnavalsa II</td>
<td>AP</td>
<td>medium</td>
<td>medium sized with eight ridges</td>
</tr>
<tr>
<td>Pot sapota</td>
<td>-</td>
<td>-</td>
<td>Fruits at a very early age, small oval fruits exceedingly sweet with good flavour</td>
</tr>
<tr>
<td>Gavarayya</td>
<td>AP, TN</td>
<td>-</td>
<td>small 8-10 ridged, flesh soft, melting and very sweet</td>
</tr>
<tr>
<td>Thagarampudi</td>
<td>TN</td>
<td>-</td>
<td>medium sized, round or oval, skin thin, flesh is buff coloured, streaked, melting and juicy, sweet</td>
</tr>
<tr>
<td>Ayyangar</td>
<td>TN</td>
<td>-</td>
<td>Rose scented, large size, round, flesh pinkish, sweet, skin thick</td>
</tr>
</tbody>
</table>

(MH- Maharashtra; TN - Tamilnadu, KA - Karnataka; GJ - Gujarat; AP - Andhra Pradesh, WB - West Bengal)
A good table sapota should have a limited seeds with melting, sweet pulp. Thick skinned, hard fleshed cultivars with sandy texture are considered inferior. There are several cultivars of sapota in India. Some of them are given in Table-1.3.

Sapota is a hardy tree and can be grown on a wide range of soils. Drainage is most important. It can be grown from sea level up to 1200 m. It prefers a warm and moist weather and grows in both dry and humid areas and coastal climate is best suited.

**Propagation**

Sapota is propagated by both seed and vegetative methods. In the earlier days seedlings were used for planting but they had some disadvantages, such as slow growth, very long pre-bearing age (8-10 years), growing to a huge size or height and showing too much of variation or not being true to type.

Vegetative methods has many advantages such as being true to type, earliness in bearing, dwarf or easily manageable size of the trees and favourable root stock may influence and improve bearing. Among the vegetative methods, the most important are air layering, ground layering, pot layering, inarching and budding. Recently attempts have been made to multiply this plant through tissue culture also.

**Air layering**

Sapota is a difficult to root plant. Many factors are known to affect the rooting ability of this plant. Vigour of the shoot has been found to be one of the important factors in this regard. The loss of rooting ability over a period of change from juvenility to maturity is a recognised fact. In rooting of air layers in sapota, the invigorated shoots produced as a result of beheading a grafted tree possess a better rooting ability than mature shoots. This method is most common in Maharashtra, Gujarat, Karnataka and Andhra Pradesh. In this method it is possible to get a sizeable plant in a short time but mortality is high and the root system is shallow. Such trees are likely to be uprooted when heavy winds are experienced in sandy soils. In Maharashtra it is believed that air layered plants give more granular fruits and that some cultivars don't strike roots easily. The other methods of layering (ground layering and pot layering) are not commercially used.

**Grafting / Budding**

Use of proper root stock is important in grafting or budding. Root stocks becomes a limiting
factor for rapid multiplication of the desirable forms in large numbers as the seedling of *A. sapota* are slow growing. The use of other related plant species as root stock poses certain problems such as incompatibility and undesirable or adverse effects. Often the incompatibility affects the fruit quality after several years. The different root stocks used are:

- Sapota seedlings (*Achras sapota*)
- Rayan or Khirni (*Manilkara hexandra*)
- Adam’s apple (*Manilkara kauki*)
- Mahua (*Madhuca latifolia*)
- Mee tree (*Bassia longifolia*)
- Star apple (*Chrysophyllum cainito*)

Sapota seedlings take a long time to attain a suitable size for grafting. In Andhra Pradesh, Adam’s apple is used as a root stock. In Srilanka, Mee tree is used as root stock. When Mahua tree is used as root stock, the plant produces fruits which are poor in quality due to the presence of an alkaloid saponin. Of all the root stocks Khirni (*Manilkara hexandra*) has been found to be the most suitable root stock for sapota. It however show variability in the rate of growth of seedling. Some types are vigorous growing, while others are comparatively slow growing. It is a more compatible root stock than Mahua. The results of root stock trials on sapota indicated that sapotas on rayan root stocks were far superior to those raised on sapota seedlings and from air layers. In terms of yield also the trees grafted on rayan gave 50% more than that on air layers and twice that on sapota seedling stock. The trees on rayan stocks were found to be healthy and strong even after four decades. Comparatively longer time for rooting and high percentage of mortality in layer necessitate resorting to grafting which is the most popular and commercial method of propagation of sapota. Some cultivars don't strike roots easily by gootee or layering and hence have to be propagated by grafting.

**Inarch grafting**

It is the commercial method of propagation practised over 40 years. Various root stocks such as sapota seedlings, rayan etc are used. The root stocks are raised in pots. The scion remain attached to the parent tree till the union is complete and if the scion branches are high, the stock plants are placed on bamboo platform or any other such devices. Side grafting and budding are also practised for successful vegetative propagation (Sulladmath & Reddy 1990).

**Manilkara hexandra**

*Manilkara hexandra* (Roxb.) Dub., (family - Sapotaceae) known as khirni (Hindi) Rayan (Gujarati) is a medium to large size evergreen tree with a spreading crown and straight massive bole found
in central India and Deccan peninsula (Fig. 2b). It is cultivated throughout the greater part of India for its yellow coloured sweet fruits (Anonymous 1962). The bark is dark grey, deeply furrowed, leaves elliptic, ovate or oblong, coriaceous, flowers solitary or in fascicles, white or pale yellow, fruits berry, ovoid or ellipsoid, 1.5-2 cm long, reddish yellow, seeds one rarely two, ovoid, 1-1.5 cm long reddish brown, shining.

*Manilkara hexandra* is a common tree in dry evergreen forests of Deccan, especially on sand stone. In very dry situation it becomes stunted and even shrub like. The tree is a light demander and natural reproduction by seeds does not ordinarily take place under dense canopy. Therefore, the best way is to girdle the trees adjoining the seed bearers. Even in such gaps made, seedling growth is poor. The flowering time of the tree is usually from November to January and the fruits ripen from April to July.

*M. hexandra* yields a strong dense timber. The sapwood is pale reddish to brownish white, sharply defined, heart wood is red to light purplish brown with darker lines when freshly cut, turning dark red on exposure, smooth, fine textured, hard, tough, strong and heavy. The timber is very durable even in contact with water, resistant to termites and needs no antiseptic treatment. It is difficult to saw when seasoned. It works to a smooth surface and takes a good polish. It is commonly used for sugar mills and oil presses, piles, posts, joists and beams in construction and agricultural implements. It is suitable for mallet heads, rollers, railway keys, brake block, tool handles, furnitures, panels and for such other articles where toughness and hardness is required.

Ripe fruits of the tree are eaten fresh or dried. They are sweet but astringent. The fruits contain moisture 68.8%, protein 0.48%, fat 2.42%, carbohydrate 27.74%, mineral matter 0.75%, calcium 83 mg, phosphorus 17 mg, iron 0.92 mg, carotene 675 I.U., thiamine 70.33 μg, riboflavin 77.41 μg, nicotinic acid 0.66 mg and ascorbic acid 15.67 mg. Seeds on extraction with ether or light petroleum yield 24.6% of an edible oil commonly known as Rayan Oil. Oil is pale yellow in colour. The seed oil is considered demulcent and emollient. The seeds contain a bitter saponin which is left in the cake after extraction of oil. Many alkaloids have been isolated from saponin. One of them is sapogenin basic acid (C_{30}H_{46}O_5 - M.P. 319°C). The bark contains 10% tannin that may be used for tanning purposes. The bark is also used in fevers and as a general tonic. It retards the fermentation of Toddy. The leaves are used as cattle fodder. The average composition of leaves is - crude protein 9.3%, ether extract 6.2%, N-free extract 53.9%, crude fibre 23.3%, total ash 74%, inositol ash 0.8% and calcium 2.0% (Anonymous 1962).

Another important use of the saplings of the plant is as a good root stock for *Achras sapota* (Chiku) of the same family. The methods usually employed are inarching and side grafting.
Manilkara has a good graft union with the scion of Achras sapota. Results of root trials of sapota indicated that sapotas on rayan root stocks were superior in yield and growth rate compared to air layers or grafts on sapota seedlings.

Conventionally *M. hexandra* is propagated through seeds (Jadhav et al 1996, Patel et al 1996, Rajput et al 1996) and stem cuttings during rainy seasons. But there are certain problems which makes the conventional methods laborious and expensive. They are

- viability of seeds is short spanned
- the germination frequency of seeds is very low in field
- the seedling growth is very slow
- irregularity of good seed year
- cuttings are not efficient under varied climatic conditions
- large scale removal of seeds by animals

As Aonla, Chiku and Khirni are economically and commercially important and are seems to be highly recalcitrant for *in vitro* culture, studies have been undertaken in these plants with the following objectives.

To standardise protocols for

(i) micropropagation via axillary bud proliferation using seedlings, grafted plants and mature tree derived explants of Aonla

(ii) large scale multiplication using low cost liquid propagation system

(iii) organogenesis from differentiated tissue by direct (direct from explants without intervening callus phase) or indirect (through callus) methods from various explants and its histology

(iv) direct somatic embryogenesis in Aonla

(v) large scale production of synchronous somatic embryos and its maturation in liquid culture system

(vi) micropropagation of two sapotaceae members- Achras sapota and Manilkara hexandra and anther culture of Achras sapota.