Chapter 7
Litchi Breeding for Genetic Improvement

N.B. Sarin, U.S. Prasad, Manoj Kumar, and S. Mohan Jain

7.1 Introduction

Litchi chinensis Sonn. ranks (high) among the most important horticultural crops, belongs to the family Sapindaceae and widely grown in tropical and subtropical regions (Menzel 1985). The tree produces delicious top quality fruits that are in great demand for their wholesome taste, sweet aroma and attractive colour. They are mainly consumed as a fresh table fruit worldwide but in China, dried litchis, called litchi nuts with the taste of the raisin are quite popular. They are also preserved and canned in syrup or used as squash. In Florida, frozen fruits are consumed on a limited scale. China, India and Taiwan are the major producers of litchi whereas in the last 40 years substantial increase in production in South Africa, Australia, Thailand, Vietnam, USA and Israel has led litchis to become a significant commodity in the international trade, (Underhill et al. 1997). Major thrust of litchi research has been on prevention of physiological browning and retention of bright red colouration of litchi fruits in several countries.

Exotic litchi fruits have received worldwide attention. Increase in popularity has necessitated litchi cultivation in a wide range of environmental conditions. In fact litchi is generally adapted to various soil types via alluvial sands, loams, heavy clay, organic soil and calcareous soil with 30% lime and rock files (Chapman 1984a). In China, the best litchi trees are prevalent in Gwanagdong province close to the rivers on alluvial sands with good drainage and access to the water table (Chapman 1984b). They are also grown in gravelly sandy loam to loam soils as well as in swampy areas. However, soil in Fijian province is very high in clay, poorly drained and acidic in reaction (Winks et al. 1983). In South Africa, trees are more vigorous in growth on acid soils rather than on neutral or alkaline soils (Marloth 1947). Under Indian conditions, litchi cultivation in Bihar state is common on calcareous soils containing more than 40% free calcium carbonate and trees flourish well in a moist subtropical climate and in deep loamy soil with high moisture content. However,

N.B. Sarin (✉)
School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India
e-mail: neerasarin@rediffmail.com

S.M. Jain, P.M. Priyadarshan (eds.), *Breeding Plantation Tree Crops: Tropical Species*,

it has increasingly gained popularity in the Bihar plateau (Kumar 1977) and sub-mountain region of Punjab (Singh and Sarin 1957). A sandy loam or clay loam with pH 5.5–7.0 having considerable soil depth and minimum of 1.5–2 m down water table with non-stagnating water is considered optimum for the growth of litchi trees.

Litchi exhibits appropriate growth in a climate characteristic of the area of its origin. Frost in winter and dry intense heat in summer are detrimental to litchi cultivation although periodic cold snaps in winter between 1 and 5 °C appear to be essential for fruit bearing. However, total duration of low temperature rather than its frequency or time of occurrence is an important factor for fruit formation (Young 1970). In Australia, plant growth is optimum at 30 °C and ceases below 15 °C but flowering occurs at 10 °C. In India, maximum temperature during flowering varies from 21 °C in February to about 38 °C in June (Pandey and Sharma 1989). Wet spring/summer and dry winter conditions are desirable for litchi fruiting in India (Kanwar et al. 1972a). China receives an annual rainfall of 1,500 mm having 69–84% humidity in litchi growing areas that is beneficial for the crop. Sunlight intensity also plays detrimental role in litchi cultivation because intense light in summer causes sun-burning and skin cracking of fruits (Kanwar et al. 1972b).

It is obvious that different soil and/or climatic conditions cover different areas of litchi cultivation. Certain elite cultivars, grown through ages in a specific area, are well adapted to soil and climate of that area. Their propagation in other areas has not been beneficial because the quality of the same cultivar is often affected. For example, ‘Shahi’ litchi of Muzaffarpur in North Bihar, producing the best quality fruits in India, is not able to retain the same quality grown in other areas of Bihar or outside. Besides North Bihar (Ray et al. 1985), the performance of litchi cultivation in other areas varies with agro climatic and soil conditions in Punjab (Jawanda and Singh 1977). Similarly the Chinese recognised the extreme difficulty of maintaining the yield of highly prized cultivars under climatic, soil or cultural conditions other than those of the tree’s original areas of selection (Menzel and Simpson 1987). Thus there is considerable variation in fruit quality that is specific to locality, environmental conditions and cultural operations.

7.1.1 Major Producers

China is by far the largest litchi producing country in the world, with the cultivated area more than 584,000 ha with an annual production of 958,700 metric tons. The most important fruit producing city is GaoZhao (in West Guangdong) near Guaghou (Canton), the very heart of South China on the banks of the Pearl River having boundless expanses of litchi plantations with beautiful dark green dome-shaped canopies (FAO 1990). Other litchi growing areas are Dongguan and Shenzhen, famous for the elite litchi cultivars like the Smile of the Emperor’s Concubine (Fexixiao), Guiwei and Nuomici. Until the year 2001, the growing areas exceeded 584,000 ha. Commercial activity is concentrated in Guangdong, Guangxi, Fijian and

Hainan Provinces with minor share from Sichuan, Yunnan and Guizhou (Huang et al. 2005).

After China, India is the second largest producer of litchi, cultivated area 56,538 ha with an annual production of 474,000 metric tons. According to State Horticulture Department, at least 1,000 ha of cropping areas are added annually in the Bihar State that contributes 77% of total litchi production in India. Tripura, Punjab, West Bengal, Arunachal Pradesh and Karnataka are other litchi producing states of India. The main bulk of the fruit production comes from China, India and Taiwan but it is gaining popularity in several other countries like South Africa, Australia, Malaysia, Burma, Hawaii, USA, Israel, Mauritius and West Indies (Pandey and Sharma 1989). The quality of litchi exported from India is considered to be the best (Table 7.1a and 7.1b).

7.1.2 Consumption

Litchi fruits are largely consumed fresh locally in the areas of production. As their shelf life is very poor, their transport to distant places face an uphill task due to rapid

Table 7.1a Production of litchi in India

Year	Area (ha.)	Production (tons)	Yield (ton/ha.)
1970	1,330	5,320	4
1975	1,876	8,629	4.5
1980	2,522	11,349	4.5
1985	2,702	16,212	6
1990	6,045	36,270	6
1995	7,088	44,643	6.29
1998	7,288	44,653	6.12
1999	7,467	43,804	5.86
2000	7,467	61,000	8.02
2001	7,517	74,000	10
2002	7,667	53,000	6
2003	7,667	75,000	9.7

Table 7.1b Litchi fresh fruits exports

Year	Quantity (tons)	Importing country
1994	10	England
1995	30	France, The Netherlands
1996	Nil	Lack of quality litchi
1997	20	England, France
1998	20	England, France
1999	30	England, France
2000	25	England, France
2001	53	England, France, Dubai(Gulf Country)
2002	42 Litchi Fresh 50 Litchi Juice	Dubai, England Nepal
2003	46 Litchi Fresh 170 Litchi Juice	England, Spain, The Netherlands, France, Nepal

deterioration in bright colouration and flavour within a few days. Several attempts to retain fruit quality even for 7–10 days have not been successful leading to expensive wastage and often resulting in a glut in the market. A good proportion of fruits undergo rotting in India. However, in China dried litchi fruits (as dried nuts) are quite popular and also exported on a large scale to several countries. Canned litchis or flavoured squash or frozen fruits are other modes of preservation and consumption which helps in avoiding a glut in the market and in preventing rooting of the fruits. Frozen litchi fruits retain flavour and quality if the freshly harvested fruits are rapidly cooled and maintained at 25 °C at which they remain in good condition for 12 months (Morevil 1973). If preserved as pulp, they are acceptable for 6 months at 25–35 °C and up to 12 months at 4−5 °C in India (Sethi 1985).

7.1.3 Uses of Litchi

Litchi fruits are among the most delicious having high nutrition and medicinal values. Edible portion of the fruit is a thick, translucent juicy aril with high sugar content that may be as high as 18–20% in elite cultivars (Chadha and Rajpoot 1969), and (Chan et al. 1975). The fruit is an excellent thirst quencher and reported to serve as a tonic for brain, heart and liver (Syamal and Mishra 1989). From the seeds of litchi, ά-(Methylenecyclopropyl) glycine, an analogue of hypoglycine A was isolated that exhibited hypoglycemic activity in animals (Gray and Fowden 1962). In China, litchi leaves are used for making poultices, the seed for skin disease and decoctions of flowers and the bark and the roots for throat gargle (Pandey and Sharma 1989). Besides possessing high sugar content litchi is a good source of ascorbic acid that ranges from 40 to 90 mg/100 g and minerals (0.7%) like calcium, phosphorus and iron (Watt and Merril 1963).

7.2 Origin and Domestication

Litchi, one of the most precious fruit crops, originated in the southern parts of China where it has been cultivated and owes its origin in the Chinese provinces of Kwantung and Fukien (Tao 1955; Ochse et al. 1961). In the words of de Candolle (1909), ‘Chinese knew about litchi only late in the third century of our era’. Though questionable, the first reference to this fruit in literature probably appeared as early as 1766 B.C. However, a clear reference has been mentioned in the literature of the Han dynasty (140–86 B.C.). A monograph by Ts’a’ Hsia ng (A.D. 1059) is possibly the first publication on litchi (Groff 1921), which is a most complete book on litchi in English. Apparently it reached Burma and eastern India by the end of the seventeenth century or shortly thereafter (Hayes 1957). Subsequently it made its way to Bengal by the end of the eighteenth century (Goto 1960; Liang 1981) from where it diversified to other parts of India and was cultivated on a commercial scale.

The introduction of litchi to several other countries has been a later event. In Hawaii, it was probably introduced in 1873 and met with remarkable success. In West Indies its cultivation started by 1775 and in Natal (South Africa) in 1869 (Marloth 1947). Although introduced in Queensland (Australia) as early as 1854, its cultivation on commercial scale was of late occurrence (Batten and Lahav 1994). In the USA it reached from Saharanpur (India) to Florida in 1883, in California in 1897 and subsequently again to Florida from Fukien province of China in 1906. The variety was named as 'Brewster' litchi. Early in the nineteenth century it also reached England and France but failed to establish (Pandey and Sharma 1989).

7.3 Botanical Aspects and Systematics

L. chinensis (Goertn.) Sonn. belongs to the family Sapindaceae, under sub-family Napheleae. Several synonyms are known namely, *Euphoria litchi* Desf., *E. sinensis* Gmel., *E. punicea* Lamk., *E litchi* Juss., *Dimorcarpus lychi* Lour., *D. litchi* Will., *Nephelium dimocarpus* Hf. and T., *N. duriocarpus* T., *N. litchi* Camb., *Sapindus edulis* Ait., *Scytalia chinensis* Goertn, *S.litchi* Roxb, *S. locacon* Roxb. and Litchi *litchi* Britton. Although belonging to the soapberry family, Sapindaceae contains 1,250 genera and about 1,000 species, mostly consisting of tropical and subtropical fruits; only a few plants can truly be termed as horticultural crops. Among them, litchi is the most important while other members are longan (*Euphoria* longana Lamj.), rambutan (*Nephelium* lappaceum L.) and pulsan (*N. mutabile* Blume). Some other species of less horticultural interest but related to litchi growing outside India are *Blighia sapida* Koen, *Melicocca bijuga* L., *L. phiippinensis* Radlk., *Pseudonephelium fumatum* Radk. and various species of Euphoria (Groff 1921). *L. chinensis* Sonn. has various common names, namely, litchi, lychee, lici, li-ci, leechee etc. (Hayes 1957). In Thailand it is called lin-chi while Malayan names are kalenkeng, lingking, laichi etc. (Allen 1967). In China it is termed as lizhi (Paull and Chen 1987). Litchi tree is a mycorrhizal round topped, 10–15 m high, evergreen tree with spreading branches. Leaves are petiolate, exstipulate and paripinnately compounds having 2–4 pairs of 8–15 cm long leaflets that are coriaceous, elliptic and oblong to lanceolate, shortly acute, glabrous and shining above. Flowers grow in terminal panicles and are polygamous, regular, small and inconspicuous having greenish white or yellowish colour. Sepals are small and valvate. Petals are often wanting. Stamens are usually eight with hairy filaments that are incurved in bud but straight, erect and far exerted later. Ovary is two-lobed and compressed silky, only one lobe usually develops into fruit. Stigma is bilobed. Fruits grow in loose bunches of 2–20. Each fruit is oval in shape, about 3.8 cm in diameter. Ripe fruits possess dry, brittle, tubercled pericarp. The colour of the ripe fruit, which is likened to a large strawberry in appearance, is rose – red to deep red and the colour changes to dull brown as the fruit dries. Botanically, the fruit is a nut but in possession of a white, translucent, fleshy and juicy aril (the edible part) developing from the funicle

and surrounding the seed at maturity makes it fleshy. The aril is of firm texture with sub-acidic flavour. On drying, the aril shrinks away from the thin outer shell (the pericarp) remaining as a rather tough structure around the seed and possess a flavour of raisin, bearing no resemblance to that of a fresh delicious ripe fruit (Pandey and Sharma 1989).

7.4 Cytogenetics

Reports on the cytogenetics of litchi have been scanty (Liu 1954; Chapman 1984b). This aspect has not received the proper attention it deserves with an aim to evolve new cultivars. The species probably originated through hybridisations of more than one wild progenitor. Haploid chromosome numbers 14, 15, 16 and rarely 17 were reported and variable chromosome number pointed to multiple progenitor origin. Liu (1954) considered the so-called 'mountain litchi' with inferior fruit quality clearly distinct and more resistant to frost than the elite cultivars existing today.

7.5 Reproductive Biology

In litchi floral axis is of compound racemose type but the flowers occur in cymes. On the basis of sex, groups of flowers in a cyme have been different and six types of cymes are noted. They are: (i) staminate flowers (ii) pistillate flowers (iii) terminal staminate and lateral pistillate flowers (iv) terminal pistillate and lateral staminate flower (v) terminal staminate with lateral flowers of different sexes and (vi) terminal pistillate with lateral flowers of different sexes. Three types of flowers are present in different branches of panicles on the same tree. They are: (a) hermaphrodite with abortive ovary, that is, functionally male; (b) hermaphrodite with non-dehiscent anthers, that is, functionally female and (c) male (Singh and Dhillon 1983). The duration of anthesis is usually between 20 and 45 days (Chadha and Rajpoot 1969; Pivovaro 1974). However, in Punjab (India) anthesis has been reported to continue for 11–17 days with optimum anthesis in the forenoon (Singh and Dhillon 1983). Anther dehiscence has been reported to begin the day after anthesis and continue up to 3 days but all the anthers do not dehisce simultaneously (Chaturvedi and Saxena 1965). In dry condition, pollen grains are barrel-shaped and tend to be triangular when mounted on a slide in water or lactic acid (Banerjee and Chaudhuri 1944). They are bi- nucleate at the time of shedding. Pollen grains of male flowers are less viable than from anthers of hermaphrodite flowers (Mustard et al. 1953) and in their germination on artificial media supplemented with sucrose, auxin and boron (Shukla et al. 1978).

The flowers are bicarpellary syncarpus with a bilobed superior ovary. The fertile lobe rapidly increases in size and turns erect and the other lobe is usually abortive. Style remains erect between ovary lobes having terminal bifid stigma with revolute branches. There is one anatropous ovule in each locule of the ovary. Functionally, pistillate flowers bloom. At the time of its lobe initiation stigma becomes receptive

exhibiting 75% (maximum) receptivity usually a day after anthesis that continues up to two additional days (Chaturvedi and Saxena 1965). Litchi flowers are self-sterile. Nectary glands are present and pollination is entomophilius. Butcher (1957) recorded 27 species from Florida, USA. *Callitroga macellaria* (a screw worm of the dipteran order) was observed to be the most effective pollinator whereas *Aphis dorsata*, the honey bee and Coleoptera, Hemiptera, Homoptera and Lepidoptera are other effective insects. In India, *Apis* spp. and *Melipona* spp constitute 98–99% of total pollinators (Pandey and Yadava 1970).

Several reports on fruit development of various litchi cultivars are available (Gaur and Bajpai 1978). One of the two locules of the ovary develops into a fruit, the other locule being shriveled and persistent as an appendage at the base of the fruit. Occasionally both locules develop into fruits. Prasad (1977) observed both locules developing into fruits in small proportions in litchi cv. 'Deshi', 'Kasba' and many other cultivars growing at Sabour, Agricultural College garden in Bihar, India. Small, immature fruits have a green velvety appearance that later turn into tubercles (epicarp tissue) at maturity (Banerjee and Chaudhuri 1944; Pandey and Sharma 1989). Within the fruit a single seed develops from an anatropous ovule after fertilisation. At the beginning, the seed is small and light green in colour that develops into a large chocolate colour at maturity. During the course of maturation, an aril emerges from the base of the seed (Huang and Xu 1983) and gradually surrounds the entire seed in fully mature fruit. The fleshy aril is translucent, white and becomes juicy with the advancement of fruit ripening (Prasad 2000).

Litchi seed is recalcitrant in nature and sensitive to moisture stress (Chin et al. 1984, Ray and Sharma 1989, Fu et al. 1990; Kumari-Singh and Prasad 1991). Within the fruit, seed viability is maintained but is rapidly lost within a day or two after separation from the whole fruit (Xia et al. 1992a and b; Prasad et al. 1996). Seeds sown soon after separation from the whole fruit, germinate readily and the optimum conditions required for germination are sand bed under shade with regular irrigation. In a litchi cultivar, 'Early Bedana' with a chicken tongued seed, traditionally considered non-viable (Pandey and Sharma 1989), germination rate was as high as 60% when sown in the sand bed under the shade (Prasad et al. 1996).

7.6 Fruit Growth Characteristics

Several reports have appeared on litchi fruit growth. Kanwar et al. (1972a) studied fruit growth at Guardaspur (Punjab, India). Singh (1977) as well as Gaur and Bajpai (1978) reported some aspects of development in a few litchi cultivars. Jaiswal et al. (1982) observed fruit growth pattern of five litchi cultivars, namely, Purbi, Deshi, Green, Ajhauli and Kasba from the orchard of Sabour Agricultural College in Bihar. They examined the whole fruit and fruit parts including the rind (pericarp), aril and the seed of various cultivars. Works of Huang and Xu (1983) as well as Huang and Qiu (1987) in Chinese cultivars 'Nuo Mi Ci', 'Huai Zhi Wei' and 'Xiang Li' recorded growth curves of whole fruit and fruit parts to be typically sigmoid,

which is consistent with earlier reports (Kanwar et al. 1972b, Gaur and Bajpai 1978; Jaiswal et al. 1982). According to these authors, the aril development depends more upon the rind than the seed coat and is repressed by a rapidly growing embryo within the seed. This explains why large seeded fruits possess a small proportion of the aril.

7.7 Conservation of Germplasm

Litchi has been under cultivation since long, but most of the commercial cultivars have been selected either in Chinese or Indian conditions resulting in their adaptation to limited climatic conditions, outside which cultivation is not as successful. Groff (1943) considered litchi to be an ecotype on account of this fact. Excessive domestication through ages poses a threat to their survival under continuously changing climate of the world. However, rapid advancement in the field of seed technology and biotechnology appears to have revolutionised the germplasm conservation in such a way that seeds or various plant parts are stored indefinitely. Cryopreservation, the technique of storage of biological material at −196 °C in liquid nitrogen is being used for long-term storage of plant germplasm that can be utilised in future breeding programmes undertaken at the National Plant Tissue Culture Repository (NFPTCR) of the National Bureau of Plant Genetic Research (NBPGR). Several laboratories in the world have standardised protocols for seed storage at −196 °C. Besides seed banks, *in vitro* gene banks have also been established in an effort towards germplasm conservation. The *in vitro* technology offers an efficient means of storing vegetative propagated recalcitrant seeds, excised embryos of which are much more tolerant to desiccation as in cases of rambutan, *Nephelium lappaceum*, closely related to *L. chinensis*, having better prospects of their storage at low temperature even cryogenically with the help of cryoprotectants (Chin and Hor 1989). Cryopreservation thus offers an alternative of seed storage to storage of tissues and excised embryos, more so in cases of recalcitrant seeds and vegetative propagated plants. In order to identify genetic materials containing useful traits for breeding and germplasm enhancement, a systematic evaluation of genetic diversity is needed for understanding relationship among accessions and their collecting site environment (Steiner and Greene 1996). Understanding the genetic diversity within a germplasm collection facilitates its use (Strauss et al. 1998). Germplasm enhancement and utilisation are important parameters for genetic improvement by breeding.

7.8 Nomenclature of Cultivars

Numerous cultivars are known throughout the world on the basis of morphological traits which have limited utility in their identification due to environmental interactions (Nielson 1985). It is but natural to have genetic markers for nomenclature of litchi cultivars that in its present state suffers from many inconsistencies, namely,

the same cultivar may be known under several names and different cultivars may appear under the same name (Aradhya et al. 1995). For instance, the cultivar 'Fay Zee Siu' of China is named 'Yu Her Pau' in Taiwan (Menzel and Simpson 1997) whereas 'Emperor', of Florida is called 'Chakrapad' in Thailand (Subhadrabandhu 1990). Isozyme analysis revealed the first two cultivars to be the same and the last two to be identical in all the enzyme systems examined (Degani et al. 1995). In fact they, for the first time, demonstrated isozyme polymorphism to be useful in proper characterisation of 30 litchi cultivars. As cultivars are ill defined, they need proper investigation.

The chief commercial cultivars are Hei Ye, Nuo Ml Ci or No Mai chee (called Groff and Hak Yip elsewhere), Huai zhi, Gui wei, Chen zi (called Brewster elsewhere), Xiang Li and Fay Zee, Siu (all Chinese cultivars); Mauritius from Israel, Chakrapad and Hang Huang from Thailand; Yu Her pau from Taiwan, HLH Mauritius from South Africa; Emperor Black Leaf Haak Yip, Bengal and Brewster from USA; and Kaimana, Sah Kang and Haak Yip from Australia. Elite cultivars of India are Shahi, Early Bedana, Late Bedana Deshi, Early Large Red Purbi Ajhauli, China, Green, Rose Scented, Dehra Rose Madrazi, Kasaili, Calcuttia, Kasba and many others (Pandey and Sharma 1989). From the overview of the traditional cultivars it is clear that their nomenclature is ill defined and without any scientific validity. For example, Aradhya et al. (1995), while investigating genetic diversity of 49 litchi accessions on the basis of comparison of isozyme finger prints, reported that some accessions identically named such as No mai tsz, Kwai mi and Hak Ip possessed different isozyme genotypes while some others with different names displayed identical isozyme genotypes. Comparison of parents using difference in DNA markers may be one of the methods with which breeders can enhance the probability of selecting those parents with different gene set. Such a method has the potential of producing progeny with new and more favourable combinations of genes for quality and yield (Kumar et al. 2006). DNA based markers like random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) therefore have come under much emphasis in order to evaluate genetic diversity of exotic litchi cultivars in several countries like China (Ding et al. 2000), Thailand (Tongpamnak et al. 2002) and India (Kumar et al. 2006). The latter found 2 out of 27 accessions, Chinarose and Late Bedana, to be genetically distant from other Indian accessions. This would be useful in mapping litchi genome and also in classical breeding.

7.9 RAPD Markers and Genetic Relationships

Compared with most tropical fruit species, genetic diversity of litchi is limited. Most varieties originated and were selected in China and are vegetative propagated. Genetic diversity in litchi is indicated in occurrence of a large number of cultivars in India and China that provide the bases for development of new

cultivars. The environment profoundly influences cultivar characteristics and this may explain why a large number of cultivars are available (Groff 1921). Litchi grown in northern parts of India, namely, states of Punjab, U.P. and Uttaranchal, where climatic conditions (low humidity, low temperature and basic soil) are different from the main litchi growing region of Bihar (India). In spite of different environmental factors litchi cultivars do not exhibit differences in flowering and harvesting time. There are different characteristics, which are used to identify the cultivars. The size and shape of litchi fruit are characteristic for different cultivars (Galan 1989). A study of 11 litchi cultivars for 8 fruit quality traits at Kalyani in the State of West Bengal (India) revealed genetic diversity and the cultivars fell into two clusters. Inter-cluster cultivar crossing might lead to heterosis for fruit trials (Dwivedi and Mitra 1995). Recently, Lin and Mei (2005) used 60 litchi cultivars, one longan cultivar and one tentative inter-generic hybrid of litchi and longan in a study of their genetic relationships and succeeded in constructing a phylogenic tree based on 470 RAPD loci amplified from 30 random primers. Their results revealed that four pairs of different cultivars, prevalent in China since long, were synonymous.

Kumar et al. (2006) reported first the genetic relatedness among Indian litchi (*L. chinensis* Sonn.) cultivars using RAPD markers. Fourteen RAPD primers that produced consistent profiles were chosen, resulting in amplification of 77 reproducible polymorphic bands. The RAPD analysis produced an average of 15.8% polymorphic and 0.10% monomorphic markers. Using the RAPD markers, all the accessions were classified into different groups despite their same or different geographical origins and climatic adaptations (Fig. 7.1). The polymorphism information content scores were calculated for each of the 77 RAPD polymorphic fragments using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Dendrograms using Jaccard's coefficients reflected no clear-cut variation or grouping based on either morphology or climatic adaptation. However, dendrogram showed that 27 accessions of Indian litchi could be classified into groups when the similarity coefficients were shown in a range of 0.11–0.47.

Two accessions (LH80 and LH109) were genetically very far distant from the other accessions using both types of markers. RAPD and AFLP marker analyses provided a quick and reliable alternative for identification of litchi accessions and determination of genetic diversity among them. Khurshid et al. (2004) reported the genetic diversity in different morphological characteristics of litchi. Genetic diversity in morphological characteristics of four litchi cultivars growing under the agroclimatic conditions of Multan was studied. Various characteristics like tree height, canopy spread, tree shape, foliage texture and colour, leaf length, width, shape and orientation, inter-nodal distance, number of leaflets per leaf, number of leaves per flush, flush colour, panicle length, number of anthers and carpels per flower, filament and style size, fruit colour and size were taken into count and variation in the characteristics was discussed. The cultivars differed in some of the morphological characteristics. The differences were probably due to their genetic make up as well as due to the influence of climatic factors. According to Tongpamnak et al. (2002) the genetic diversity and relationships within Thai litchi cultivars was

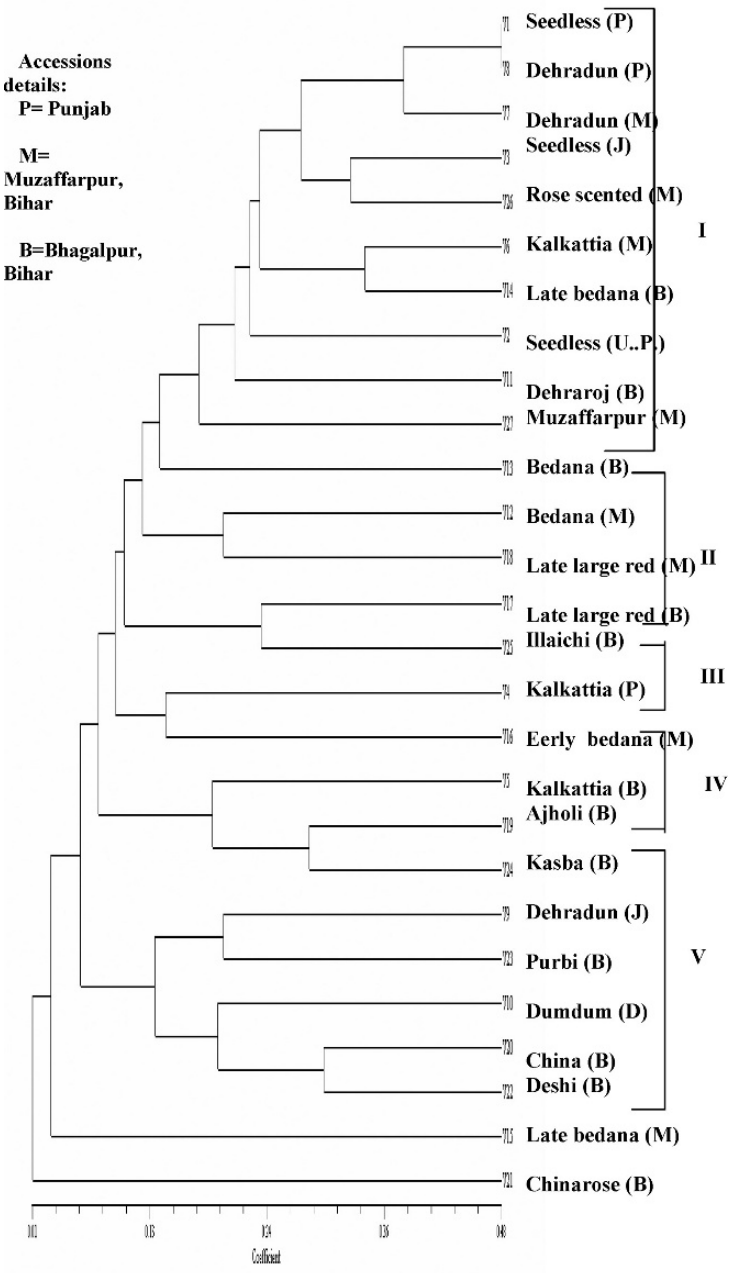

Fig. 7.1 Dendrogram derived from UPGMA cluster analysis using disc coefficient of RAPD markers

investigated using RAPD and AFLP markers. Fourteen RAPD primers and seven AFLP primers were chosen, resulting in amplification of 52 and 101 reproducible polymorphic fragment products, respectively. The percentages of polymorphic markers for RAPD and AFLP were 34.6% and 36.3% respectively. Each marker system was able to differentiate all accessions. Each of the AFLP primers could also identify all accessions, while the RAPD markers did not show such efficiency. The polymorphism information content (PIC) scores were calculated for each of the 52 RAPD and the 101 AFLP polymorphic fragments. It ranged between 0.16 and 0.50 for RAPD markers and 0.22–0.50 for AFLP markers. Anuntalabhochai et al. (1999) reported an analysis of genetic variation within 20 litchi cultivars using random amplified polymorphic DNA (RAPD) technique. Out of the 69 arbitrary primers, 5 primers named OPB18, OPC09, OPAK10, OPAQ12 and OPAS10 produced reliable DNA polymorphism ranging in molecular weight from 200 to 2000 bp. DNA patterns from RAPD data were analysed by cluster analysis and UPGMA to present a dendrogram depicting the degree of genetic relationship among the 20 cultivars. These cultivars were classified into two major groups with each containing three sub-groups. Analysis revealed that some cultivars known as O-Hia and Haak Yip displayed identical fingerprint patterns indicating that it is the same cultivar known under two different names. Others such as Kwang Jao and Jean Hom, and Haew and Luk Lai exhibited high similarity in their patterns indicating a close genetic relationship.

Ding et al. (2000) studied the segregation patterns of RAPD markers in an F_1 population of *L. chinensis* Sonn. F_1 population was established from a cross between cultivars Wuye and Luhebao of *L. chinensis* Sonn. using the pseudo testcross format. Twenty nine, 10-mer primers were screened out of 75 primers and 294 RAPD markers were amplified from 68 F_1 seedlings. Of these 53 polymorphic markers, 36 segregated in a ratio of 1:1 (band present; band absent). Among these 36 markers, 21 were present only in 'Wuye' and 15 only in 'Luhebao'; 17 markers segregated at a ratio of 3:1 (band present: band absent) and 232 markers did not segregate in F_1 (the parent genotypes may be AA × AA, AA × Aa, Aa × AA, aa × AA or AA × aa). Additionally, 7 markers deviated from Mendelian Law and two showed abnormal segregation patterns. Of the 146 markers present in 2 parents, 123 bands (84.2%) did not segregate and 17 bands (11.6%) segregated normally (3:1). Of the 148 markers present in only a single parent, 14.2% were in 'Wuye' and 10.2% in 'Luhebao'. The information obtained from the molecular marker analysis could be of practical use in mapping the litchi genome as well as for classical breeding.

7.10 Problems Facing Litchi Production

Major bottlenecks that limit fruit production are listed below and they need careful attention:

7.10.1 Lack of Quality Seeds and Genuine Plant Material

Because of long gestation period, high heterozygosity, lack of information on inheritance pattern, infection of seeds and inadequate supply of genuine and certified plant material to the growers are some of the reasons for the low productivity of fruit crops. In other perennial fruit crop like mango, plant multiplication is being done by grafting techniques on nondescript root stock resulting in inferior plants. Similarly, litchi crop is being multiplied through stooling, air layering and budding that are sluggish and cumbersome and the plants are not multiplied through elite 'mother of superior' quality. Therefore, multiplication should be done only from the mother plants of established superiority. It would be desirable to establish elite orchids of important fruit crops in the fruit growing state for the supply of authentic plant materials.

7.10.2 Constraints in Micro-Propagation

In vitro oxidative browning of cultures, contamination, vitrification and high mortality during acclimatisation are some of the problems associated with micropropagation of woody fruit trees. *In vitro* oxidative browning can be controlled using different phenol binding agents, modifying redox potential of media, quick sub-culturing, keeping cultures in the dark and through explant waxing (George and Sherrington 1984, Mishra and Mishra 1999). The conditioning of stock plant has been very important for the establishment of shoot tips and nodal explants. New vegetative growth was found to be used as an explant in jackfruit, guava (Amin and Jaiswal 1988) persimmon (Mishra 1982) and a number of other fruit crops (Amin 1992). Substantial numbers of micro-propagated plants do not survive from in vitro condition to green house or field environment. The plantlets develop within the culture vessels under low levels of light, aseptic conditions on medium containing ample sugar and nutrient to allow for heterotrophic growth and in an atmosphere with high relative humidity.

7.11 Litchi Agronomic Practices

7.11.1 Cropping Systems

A common practice in litchi orchard is pruning of young trees in order to establish a strong framework that will facilitate harvesting later. Young trees are sensitive to intense heat, frost and high winds. They require moist soil with regular irrigation for their active growth. Mature trees require regular use of fertilisers (Pandey and Sharma 1989). Trees are tinctured after completion of post-harvest flush. Maintenance of good sanitation and weed control are essential factors for keeping a litchi orchard in healthy and disease free atmosphere. Since litchi is a slow growing

tree and takes about six years to come to the bearing stage, inter-cropping of a young orchard during the vegetative period is a widespread practice in all litchi growing areas. Chaturvedi and Jha (1998) studied in detail the crop production and economics under litchi plantation across 1–9 year age series in North Bihar, India. They used inter-crops under two rotations, namely, paddy-wheat-green gram, ginger-maize, maize-mustard etc. under 1–9 years of litchi plantations.

7.11.2 Watering

Young orchards require regular irrigation otherwise plants will not be established properly and the growth is affected. Adequate watering is required at the fruit bearing stage. It is essential to maintain optimum soil moisture content (Cull and Hams 1974). However, if annual rainfall is more than 125 cm and well-distributed, irrigation may not be needed (Pandey and Sharma 1989). In India, critical period for irrigation is from January end to monsoon break (June end/July beginning). Chapman (1983) suggested that irrigation in Queensland (Australia) needs to be increased from the time of panicle emergence to the fruit harvest in order to promote floral and fruit development as well as initiation of post-harvest vegetative flush. Chandel et al. (1995) investigated the effect of irrigation frequencies on yield and quality of 26-year-old litchi cv. Rose Scented growing in Nainital (India) and reported highest yield of 78.5 kg/tree following 7 irrigations at 15 day intervals with low incidence of fruit cracking (6.69%) . In another cultivar Dehradun highest fruit set (14.36%), fruit retention (9.98%), fruit size, weight and yield (64 kg/tree) was recorded from trees sprayed with NAA (1-naphthalenacetic acid) and irrigated at 20% depletion of available soil moisture .Though litchi is a deep rooted fruit tree, absorbing roots mostly exist in the topmost layer of the soil at 20–30 cm depth that must be maintained at 50% soil moisture or above (Cull 1977). A calendar of operations all year round for irrigation and use of fertilisers besides growth regulators has been prescribed by Pandey and Sharma (1989).

7.11.3 Fertilisers

Nutritional requirements and use of fertilisers formed the basis of major thrust of an earlier research on litchi. It was established that NPK application increased fruit yield (Koen et al. 1981a and b) and their deficiency resulted in stunted growth and floral initiation. Micro-nutrients like zinc, boron and copper are very important for litchi nutrition. For plantation in the orchards, digging open pits of about 2–3 weeks beforehand and filling with a mixture of rotten farmyard manure silt is followed by filling with a mixture of farmyard manure (20–25 kg), bone meal (2 kg) and potassium sulphate (400 g) to be mixed with top soil in the pit. It is thus clear that the nutritional requirements for litchi plantation are high. Koen et al. (1981b) recommended the highest yield with 1,200 g nitrogen per tree initially to be increased

to 3,600 g nitrogen later. For mature trees a mixture of calcium ammonium nitrate (4 kg), super phosphate (3 kg) and potassium chloride (1.5 kg) per tree per year was also recommended (Koen and Smart 1982). However, in India little or no manure was applied earlier (Hayes 1957) but Yamdagani et al. (1980) recommended 1.0 kg nitrogen, 300 g phosphorus, 300 g potassium and 40 kg FYM per mature tree per year. Thus practice of fertiliser application has been different at different places. In recent years various trials of fertilisers have been undertaken in order to maximise fruit yield and rapid healthy growth of litchi trees. Field experiments on 21-year-old litchi trees were conducted and application of N, P and K at 0.84, 0.5 and 1.2 kg per year respectively increased the yield (Chen et al., 1998). At Pantnagar in India, application of 1,200 g nitrogen per tree with 300 g per year and potassium resulted in the highest yield and improvement of fruit quality (Lal and Tiwari 1996). Likewise, Sharma and Mahajan (1997) made a critical appraisal of fertiliser requirements during the years 1975–1995 at Gurdaspur (India). Hasan and Chattopadhyay (1993) observed change in growth and fruit quality of litchi cv. Bombai when put on various trials of NPK nutrition.

7.11.4 Pest Control

A considerable fruit loss (3.6%) is caused by insects and birds. Out of the 40 insect species reported (Vevai 1971), two insects, namely, eriophyid mites (*Aceria litchi*, syn. *Eriophyes litchi*) and bark eating caterpillars bring about serious damage (Butani 1977). Other important insects are scale insects, leaf miners, bugs, weevils, fruit and seed borers, etc., that occasionally infest. Eriophyid mite is widespread in litchi growing countries but its incidence is maximum in North Bihar and Mysore (India). Besides infestation of leaves, it causes inflorescence malformation (Das and Chowdhary 1958). Several control measures and insecticide sprays are in practice that has been dealt in detail by Pandey and Sharma (1989). Important bark eating caterpillars like *Indarbela quadrinotata* Walker and *I. tetraonis* More and some other bark borers are known to cause damage (Chang 1970; Villiers and Mathee 1973; Rai and Bhandary 1973). Cleaning the affected portion after removal of webs formed and plugging the holes with cotton wool soaked in carbon bisulphide, chloroform, formalin or petrol and finally with mud has been an ineffective control measure against the insect pests. Low concentrations (0.05%) of dichlorofos and p-sulfan or parathion (Villiers and Mathee 1973) are also effective. In addition to these control measures, exploitation of proteinanceous enzyme inhibitors in integrated pest management, as in other crops, appears a sound future prospect that emphasises the need for development of transgenic litchi trees resistant to insect pests. Not only genes encoding proteins and amylase inhibitor have been isolated and cloned, transgenic crops using these genes have been developed (Chrispeels et al. 1998; Ussuf et al. 2001). Hopefully this strategy can be exploited in litchi in the near future.

7.11.5 Diseases and Control

Numerous reports have appeared on incidence of diseases caused by fungal pathogens after fruit harvest in several countries (Pandey and Sharma 1989) and Underhill et al. (1997). *Collectotrichum* and *Phomopsis* sp. are reported to infect fruits in the field before harvest (Johnson and Sangchote 1994). Some of the post-harvest pathogens include several species of *Aspergillus*, *Pencillium, Botryodiplodia*, *Pestalotiopsis, Fusarium, Trichoderma* etc. Yeasts and bacteria are also reported (Roth 1963) to attack litchi. Duvenhage (1993) reported control of post-harvest decay and browning of litchi fruits by sodium metabisulphite dip followed by hydrochloric acid dip or vitafilm. Several fungicides like benomyl, prochloraz and imazalil are effective in disease control (Pandey and Sharma 1989; Underhill et al. 1997). In addition to the use of fungicides, refrigeration, heat treatment and orchard hygiene are other control measures. Post-harvest handling at low temperature minimises disease occurrence. Incidence of red rust is caused by parasitic alga *Cephaleuros virescens* on stem and leaf of litchi, causing loss of vigour due to bank canker and brown leaf felting. Gupta et al. (1997) recorded it in litchi orchards in the Kangra Valley of Himachal Pradesh (India) at an altitude of 750–950 m and infestation ranged from 50 to 90%. Four sprays of dimethioate (0.03%) or dicofol (0.05%) at monthly intervals followed by pruning and burning of affected parts is an effective control measure.

An entophytic fungus *Phomopsis litchii* has been reported and its occurrence poses problem for *in vitro* culture. This could be controlled by including bavistin, a broad range fungicide in medium bavastin (Kumar 2006). Development of disease resistance through biotechnology is a major potential area that needs to be exploitated in future for preventing expensive wastage of the plants as well as the fruit.

7.12 Breeding Objectives and Strategies

7.12.1 Breeding Objectives

Litchi is a cross pollinated crop with a high degree of heterozygosity that puts constraints on developing plants through sexual means. Most of the commercial cultivars selected under Chinese or Indian conditions have been adapted to limited climatic conditions (Pandey and Sharma 1989). Characters like fruit size, quality and period of maturity formed the basis of cultivar selection, raised through asexual means. However, in order to diversify litchi cultivation, in addition to these, other characters like precocity, dwarfness, regularity of bearing, wider adaptability tree characters and resistance to physiological disorders are of paramount importance. Raising plants through asexual means generates plants that are true to the parental types but variations are not obtained. Variations would arise only through sexual

reproduction and new variants would provide the basis for new selections through genetic manipulation. Thus there is urgent need for breeding work and raising plants through seeds. Survey of literature points to the fact that no attention has been paid to the breeding work for raising new varieties except for small selections for which programmes were initiated sporadically in Hawaii (Storey et al. 1953), Queensland, Australia (Cull 1977) and Saharanpur (India) (Lal and Nirwan 1980). As a result, important cultivars like 'Groff', and 'Brewster' were developed. Undertaking a breeding programme on a large scale needs a comprehensive survey of various genotypes and their inheritance pattern because of obvious difficulties in litchi breeding (Hamilton and Yee 1970; Menzel 1985).

7.12.2 Breeding Strategies

Different breeding strategies are applied for developing new litchi cultivars with improved traits. It takes a long time to develop a new litchi cultivar by conventional breeding. Therefore, it is desirable to reduce the breeding time in producing new improved cultivars. Breeding methods combined with new technologies including genetic engineering, *in vitro* mutagenesis and molecular assisted breeding would assist litchi breeders to develop new cultivars in a cost effective manner.

7.12.2.1 Conventional Breeding

There are many serious problems associated with raising plants through seeds, such as loss of seed viability and its short life span, slow seedling growth and long juvenile period of vegetative growth. In the last 20 years breeding programmes have been undertaken on a large scale in several countries. At the Institute for Tropical and Subtropical Plants (ITSP), South Africa, a breeding programme was initiated during 1992–1993 for the purpose of cultivar selection for South African conditions, aiming at an annual establishment of at least 1,000 seedlings for evaluation and selection (Froneman and Oosthuizen 1995). In China, breeding and selection for extension of the bearing period and better quality cultivars has been initiated (Huang et al. 2005). A seedless litchi variety produced by conventional breeding in Hainan (China) (Fig. 7.2).

In Australia, genetic improvement of litchi has been reported through reciprocal controlled crosses among several existing cultivars (Dixon et al. 2005). A majority of the seeding trees came into production within three years when planted in subtropical and tropical conditions and one notable performer produced 32 kg of fruits with individual fruit weight being 35–45 g. Four new litchi selections were recommended for cultivation in Hainan province of China. Out of these, Aili is a dwarf selection producing fruits of 24.8 g (average weight) whereas another selection Ziangxi has large fruits (39.1–595 g) of very good eating quality. Thus breeding for selection of new litchi cultivars has been receiving the attention of researchers and it is possible that breeding strategies may succeed in control of browning so that the bright red colour of the litchi fruit can be retained (Underhill et al. 1997).

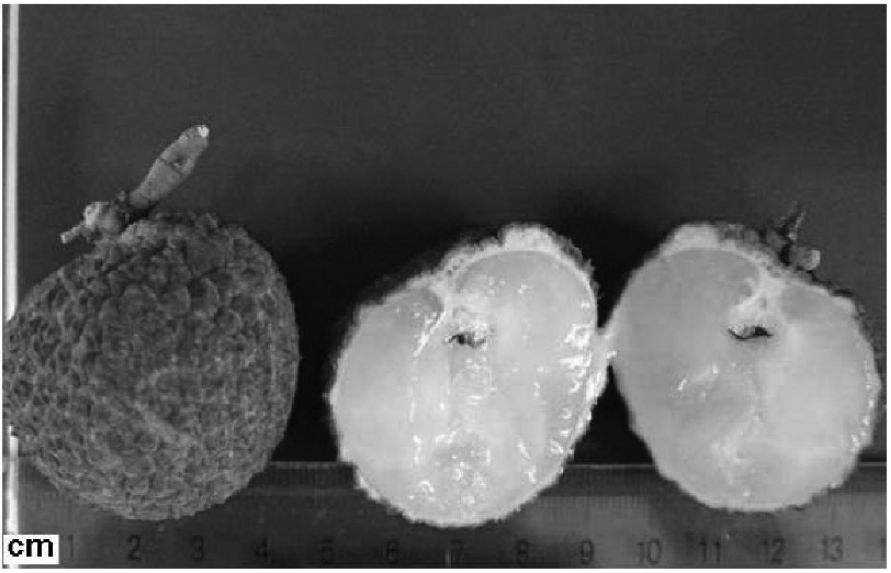

Fig. 7.2 A seedless litchi cultivar in Hainan, China (Photo provided by Prof. Wang, China) (*See* Color Insert)

Other important areas of interest are extension of the bearing period, irregular bearing, poor shelf life and disease resistance. These need to be addressed in future programmes on litchi breeding.

7.12.2.2 Transgenic Approach

There is one recent report on transformation of litchi. Puchooa (2004) reported Green-fluorescent protein (GFP) gene expression in leaf tissues of litchi after transformation using *Agrobacterium*. *In vitro* grown leaf tissues were used for transformation. After four weeks in culture, expression of GFP was apparent when the regenerated callus and the leaves were observed under fluorescence microscope fitted with a blue exciter filter, a blue dichroic mirror and a barrier filter. Although no transformed litchi plantlets were regenerated, screening for GFP gene expression may prove useful to improve transformation efficiency and to facilitate detection of transformed litchi plants. Although Ouyang and Zheng (1985) reported T-DNA transfer and tumor formation induced by *Agrobacterium tumefaciens* on litchi, genetic transformation of litchi using *Agrobacterium* could be a reality in the future.

7.12.2.3 *In Vitro* Mutagenesis

Nuclear applications in food and agriculture have contributed greatly in enhancing agriculture production of seed and vegetatively propagated crops (Jain 2005).

Even though nuclear technology has benefited agriculture greatly, it still has a great potential in genetic improvement of litchi and other crops. More than 2,600 mutant varieties have officially been released in many countries (http://www-mvd.iaea.org). Both chemical and physical mutagens are used to induce mutations. Among them, gamma rays and ethyl-methane sulphonate (EMS) are widely used for mutation induction. Fine embryogenic cell suspension cultures are most suitable for inducing mutations by transferring to the filter paper and plated on the agar-solidified culture medium for gamma irradiation. Initially LD_{50} dose is determined, which is used as an optimal dose for mutation induction. Irradiated cells are further cultured to the fresh medium for the development, maturation and germination of mutated somatic embryos. This approach provides mutated somatic seedlings in a short period and also prevents chimeras problem that otherwise requires to multiply plants up to $M1V_4$ generation for chimera dissociation. Alternatively, shoot tip or bud wood can be irradiated and multiply plants up to $M1V_4$ generation for producing pure mutants by dissociation of chimeras.

The genetic improvement of litchi for improving or developing new varieties requires genetic variation. However, the desirable genetic variation is most often lacking and that hampers the breeding of litchi. This is because the existing germplasm fails to provide the desired recombinants and it is necessary to resort to other resources of variation. Since spontaneous mutations occur with extremely low frequency, mutation induction techniques provide tools for the rapid creation and increase in variability in crop species. The genetic variability can be induced by mutagenic agents, such as radiation and chemicals, and from which desired mutants could be selected (Jain 2006). The mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism new mutations are induced randomly and heritable. The changes can occur also in cytoplasmic organelles and also result in chromosomal or genomic mutations and that enable plant breeders to select useful mutants such as flower colour, flower shape, disease resistance and early flowering types (Jain and Maluszynski 2004). A specific advantage of mutation induction is the possibility of obtaining unselected genetic variation, improvement of vegetatively propagated plants when one or few characters of an outstanding cultivar are to be modified.

7.12.2.4 Molecular Marker Assisted Selection and Breeding

As mentioned earlier, nomenclature of litchi cultivation suffers from many inconsistencies that need scientific attention (Aradhya et al. 1995). While the isozyme pattern was used as a reliable marker, rapid developments in technology led to the introduction of DNA based molecular markers that have proved to be very efficient. Among these, RAPD and AFLP, markers are quite beneficial for assessing genetic diversity. In recent years, RAPD markers have been successfully exploited in litchi (Ding et al., 2000; Tongpamnak et al. 2002; Kumar et al. 2006). Comparison of parents displaying differences in DNA markers may be useful for breeders in selection of parents with different genomes resulting in new combinations of characters in their progeny.

7.13 Propagation

Excellent reviews are available on various aspects of propagation (Menzel 1985; Pandey and Sharma 1989) including micro-propagation (Sarin and Prasad 2003; Sarin et al. 2003) in litchi. High heterozygosity and genetic diversity as a result of cross pollination put constraints in practicing propagation of elite cultivars of litchi through seeds on a commercial scale. New plants often come into fruit bearing after a prolonged vegetative phase (Hamilton and Yee 1970) and fruit quality is much inferior to their parental types (Joubert 1970; Loebel 1976). Nevertheless, seed propagation is vital for the development of new selections (Kadaman and Slor 1974) for root stocks and for breeding purposes.

As mentioned earlier, major problem associated with litchi propagation through seeds is their short life span and rapid loss of viability under desiccation (Menzel 1985; Pandey and Sharma 1989; Fu et al. 1990; Kumari-Singh and Prasad 1991). Litchi seeds may keep well within fruit up to a month but start losing viability even within a day after separation from the fruit (Menzel 1985; Prasad and Prasad 2004).Cull and Paxton (1982) earlier reported best storage of seed while inside the fruit or in moist peat moss in a freezer. Optimum seed germination was observed when seeds were sown immediately after their separation from the fruits (Prasad et al. 1996) and effect of desiccation on their germination was investigated by Xia et al. (1992a and b), Prasad and Prasad (2004).

7.13.1 Conventional Propagation of Litchi

Conventionally, litchi propagation involves asexual means to develop new plants true to the parental type (Syamal and Mishra 1984; Pandey and Sharma 1989; Menzel 1985). Air layering, grafting, budding and stolling are other means of litchi propagation. Of these, air layering is the most common and successful; it is called gootee in India. Grafting and budding are not in vogue because of non-availability of vigorously growing healthy seedlings for use as root stocks. The major shortcomings of marcottage and grafting in developing plants involved depletion of branches in large numbers from the mother trees besides being slow and inefficient (Chapman 1984). Therefore, alternative efficient breeding methods that can provide new plants true to the parental types in large numbers while overcoming the major shortcoming of asexual means of litchi propagation are the forms of research.

There is a great potential for *in vitro* regeneration of litchi. If successful, it may develop into a major industry. Selected elite cultivars need to be multiplied through cloning on a large scale evaluated at the field level and preserved on a long term basis (Sarin and Prasad 2003). Some attempts have been made in this regard although it is a difficult task considering initial difficulties in propagating through *in vitro* techniques (Kantharajah et al. 1989). However, callus induction, somatic embryogenesis and plant regeneration from immature zygotic embryos and anthers have been reported (Zhou et al. 1996; Kantharajal et al. 1992; Fu and Tang 1983).

Yu and Chen (1998) further reported development and maintenance of androgenic suspensions and protoplasts isolated from several litchi cultivars that was followed by somatic embryogenesis and plant regeneration from protoplasts isolated from zygote derived embryogenic suspensions of litchi var. xiafanzhi (Yu et al. 2000). Das et al. (1999) reported multiple shoot formation and plant regeneration from the cotyledonary nodes as well as by *in planta* treatment of the axillary bud regions. In another study, a reproducible method of in vitro regeneration of elite litchi trees for clonal propagation has recently been reported (Kumar et al. 2006).

7.13.2 In Vitro Regeneration (Micro-propagation) of Litchi

Litchi can be propagated through various asexual means such as grafting, stem cutting, air layering or marcottage and budding (Menzel and Simpson 1987; Pandey and Sharma 1989). These methods are useful in raising 'true-to-the parental type' plants and desired cultivars are properly maintained and become easily available in large numbers. However, such propagation methods involve depletion of branches in great numbers from the mother trees (Ray and Sharma 1985). Although conventional vegetative propagation methods are slow and inefficient (Chapman 1984), these methods are used under horticultural practices in many countries. Several researchers have used auxins indole butyric acid (IBA) and α-napthalene acetic acid (NAA) to promote root initiation and better root development. Genotype, physiological condition and wood type of the parent tree in addition to environmental factors are important considerations for successful development of a root system by stem cuttings (Pandey and Sharma 1989).

Grafting and budding are not in vogue primarily because of the non-availability of vigorously growing healthy seedlings of litchi for use as root-stocks. Therefore, alternative methods for raising the chosen elite cultivars must be attempted. Regeneration of litchi *in vitro* is an alternative to vegetative propagation for mass scale production of desired cultivars. This technique also spares branch depletion. *In vitro* regeneration of litchi that is a prerequisite for genetic engineering might also prove beneficial in improving the shelf life of fruits as well as enhancing the viability of seeds using appropriate genes and transformation methods.

Initial attempts towards clonal multiplication of litchi using seedlings and mature tissues failed to yield any positive results (Wolf 1987; Kantharajal et al. 1989). However, protocol for clonal propagation or direct regeneration through nodal cuttings has been established for further improvement (Kumar et al. 2006).

7.13.3 Direct Regeneration from Nodal Explants of Litchi

Micro-propagation is defined as the true-to-type propagation of selected genotypes using *in vitro* culture techniques. Depending on the species and cultural conditions, *in vitro* propagation can be achieved by the following three basic methods (Kane

1996): (a) Enhanced axillary shoot proliferation (shoot culture), (b) Nodal culture and (c) *De novo* adventitious shoot formation through shoot organogenesis.

Kantharajal et al. (1992) was the first to demonstrate a method for litchi regeneration through *in vitro* technique. This method involved the embryos of different sizes and ages from commercial varieties of litchi that were cultured in a range of different media. They reported that pre-treatment of embryos with 100 mg^{-1} BA in liquid medium for 3 hours was optimum for adventitious shoot formation. Subsequent transfer to MS semi-solid medium containing thiadizuran (1 mg l^{-1}) resulted in the formation of 5 shoots/explant in cv. Bengal. Das et al. (1999) suggested two methods of shoot multiplication. The first involved shoot bud initiation from seeds directly germinated on filter-paper bridge submerged in liquid MS medium supplemented with 20 mgl^{-1} BA. The second method (*in planta*) involved the use of 4–5 week old seedlings that had been germinated and grown on vermiculite in culture bottles. The leaf axils (nodal region) of these seedlings were treated with 100 μl solution of various concentrations of BAP ranging from 0.25 to 1.0 mg/ml that was supplemented on alternate days through a moist filter paper placed in direct contact with axillary meristem for 8 weeks. Highest number (8) of multiple shoots was observed after 7–8 weeks of BAP (1 mg/ml) treatment. Both methods of multiple shoot induction were effective for the five genotypes of litchi Chandra and Padaria (1999) cultured shoot buds of litchi cv. Seedless on MS medium supplemented with 0.2 mg BA + 0.1 mg IAA + 0.5 mg GA_3/litre and obtained shoot differentiation and growth.

Kumar et al. (2006) established for the first time a rapid regeneration system through *in vitro* culture of litchi by culturing nodal segments obtained from field grown plants. Different cytokinins were tested; explants grown in the presence of BAP, 2-iP, Kin and other additives (coconut water, casein hydrolysate, silver nitrate etc.) gave rise to multiple shoot formation from the nodal segments.

The cytokinins were effective only when provided in moderate concentrations over a period of time, while higher concentrations proved counter-productive. BAP at a concentration of 11 μM was most suitable for the regeneration from nodal explants and 6 μM BAP was the optimum for further elongation and multiplication of shoots.

Pulse treatment of IBA to the well developed shoots followed by culture in MS medium supplemented with IBA (20 μM) and litchi seed powder (1 g/l) was found to be the best rooting medium for litchi. *In vitro* grown plantlets were successfully transferred to the field and they are surviving in the harsh climatic condition of Delhi. This system is ideally suited for mass scale propagation and may also be amenable for further industrial application.

7.13.4 Somatic Embryogenesis

Somatic embryogenesis is the process by which somatic cells develop through the stages of embryogenesis to give whole plants without the fusion of gametes. Somatic embryogenesis was defined by Emons (1994) as the development from somatic cells of structures that follow a histodifferentiation pattern that leads to a body pattern

resembling that of zygotic embryos. *In vitro* somatic embryogenesis can either occur directly from callus or suspension culture (Williams and Maheswaran 1986).

7.13.5 Induction of Embryogenic Callus and Histological Study

Somatic embryogenesis has been attempted in litchi. Zhou et al. (1996) reported that MS medium supplemented with 2.0 mgl^{-1}2, 4-D + 0.2 mgl^{-1} BA + 0.12 mgl^{-1} NAA was ideal for embryogenic callus formation from immature embryos (20-, 30- and 50-day-old) of 4 varieties of litchi, although rapidly growing calli were also obtained on MS medium containing 4.0 or 6.0 mgl^{-1} 2, 4-D + 0.1 mgl^{-1} BA + 0.1 mgl^{-1} NAA. Somatic embryo development and complete plantlets were obtained on MS medium supplemented with low concentration of NAA and IBA. Yu and Chen (1998) reported the induction of litchi (Xiafanzhi) embryonic calli from immature embryos and anthers cultured in vitro. The immature zygotic embryos were removed and transferred onto induction medium (MS1) that included MS salts B_5 vitamins, 50 gl^{-1} sucrose and 2 mgl^{-1} 2, 4-D. The cultures were maintained in darkness and the embryogenic callus that appeared after 6–8 weeks of culture was pale yellowish and friable. Yu and Chen (1998) utilised this callus to generate embryogenic suspension culture. Liao and Ma (1998) carried out a thorough investigation of somatic embryogenesis and plantlet regeneration in litchi *cv*. Yuherbau. Secondary embryos appeared in large numbers on the surface of immature primary somatic embryos in a culture medium containing 0.05 mg/l NAA, 0.05 mg/l 2-ip, 0.2 mg/l ABA. According to several researchers (Merkle 1995; Yu et al. 2000), culturing on medium supplemented with 2, 4-D followed by callus growth onto the medium devoid of 2, 4-D gave rise to somatic embryos and eventually to the plantlets. Histological studies of somatic embryos of different species have been described for both pathways of origin: unicellular and multicellular (Vasil and Vasil 1982; Alemanno et al. 1996).

7.14 Conclusions

Mutagenesis approach would be ideal for developing new mutant lines and also for enhancing germplasm of litchi. International Atomic energy Agency (IAEA) maintains mutant variety database (www.iaea.org) that includes over 2,600 officially released mutant varieties of various crops in different countries. A wide range of mutants would be of great use in molecular characterisation and identify trait specific molecular markers. These markers will be helpful in molecular marker assisted selection and breeding.

Plant regeneration of litchi through *in vitro* techniques on a mass scale would require more research as this area is still in its infancy. Vegetative propagation of litchi maintains 'true-to-type' nature of cultivars as propagation through seed would produce inferior cultivars in terms of fruit quality, size and maturity period. How-

ever, diversification of litchi cultivation also demands other characters like precocities, dwarfness, regularity in bearing wide adaptability and resistance to disorders need to be considered. Asexual means of propagation cannot provide variations needed for such purposes whereas sexual means produces a large number of variants. Thus there is an urgent need for raising plants through seeds because now variants would provide better selections.

In the last 20 years breeding programmes in litchi had been undertaken on a large scale. For instance, South Africa aims at 100 seedlings for evaluation and selections annually. In China, breeding and selection for extension of bearing period and quality improvement have resulted in four new selections including 'Aili', a dwarf selection and 'Ziangxi' with large fruits (39.1–59.5gm). In Australia, genetic improvement through reciprocal controlled breeding among several existing cultivars gave rise to new seedlings. With shortened vegetative phase, fruit production starts within 3 years of plantation.

Breeding for selection of new cultivars has immense potential and holds a promising future in tackling problems of browning, prolongation of self-size and extension of the breeding period of the litchi.

Further advent of new technologies involving DNA-based molecular markers like 'RAPD' and 'AFLP' are quite useful in assessment of genetic diversity, benefiting the breeders in parent selection and new combinations of characters in their progeny.

Acknowledgments DBT grant number BT/PR/1775/AGR/8/122/99DT 1/76/2000 to NBS is gratefully acknowledged.

References

Alemanno, L., Berthouly, M., and Michaux-ferrière, N.A. (1996) Histology of somatic embryogenesis from floral tissues of cocoa. Plant Cell, Tissue Organ Cult. 46, 187–194.

Allen, B.M. (1967) *Malayan Fruits: An Introduction to the Cultivated Species*. Donald Moore Press, Ltd., Singapore.

Amin, M.N. (1992). In vitro rooting of jackfruit (*Artocarpus heterophyllus*) microcuttings derived from mature trees. Plant Tissue Cult. 2, 129–133.

Amin, M.N. and Jaiswal, V.S. (1988) Micropropagation as an aid to rapid cloning of a guava cultivar. Scientia Hort. 36, 89–95.

Anuntalabhochai, S., Chundet, R., Chiangda, J., Apavatjrut, P. (1999) Genetic diversity within lychee (*litchi chinensis* sonn.) based on RAPD analysis International Symposium on Tropical and Subtropical Fruits: ISHS Acta Horticulture: 575.

Aradhya, M.K., Zee, F.T., and Mandshardt, R.M. (1995) Isozyme variation in lychee (*Litchi chinensis* Sonn.). Sci. Hortic 63, 21–35.

Banerjee, I. and.Chaudhuri, K.L (1944) A contribution to the life history of (*Litchi chinensis* Sonn.). Proc. Indian Acad. Sci., Section B: 19–17.

Butani, D.K. (1977) Pests of litchi in India and their control. Fruits 32, 269–273.

Batten, D.J. and Lahav, E. (1994) Base temperatures for growth processes of lychee, a recurrently flushing tree, are similar but optima differ. Australian J. Plant Physiol. 21, 589–602.

Butcher, F.G. (1957) Pollinating insects of lychee blossoms. Proc. Florida State Hort. Soc. 70, 326–328.

Chadha, K.L. and Rajpoot, M.S. (1969) Studies on floral biology, fruit set and its retention and quality of some litchi varieties. Indian J. Hort. 26, 124–129.

Chan, H.T. Jr., Kwok, S.C.M., and Lee, C.W.O. (1975) Sugar composition and invertase activity in lychee. J. Food Sci. 40, 772–774.
Chandel, S.K., Kumar, G., and Kumar, G. (1995) Effect of irrigation frequencies and folier spray of NAA and micronutrient solutions on yield and quality of litchi*(Litchi chinensis* Sonn.) cv. Rose Scented. Adv. Plant Sci. 8, 284–288.
Chandra, R. and Padaria, J.C. (1999) Litchi shoot bud culture for micropropagation. J.Appl. Hort. Lucknow, 191:38–40.
Chang, K.P. (1970) A Study of *Anoplophora macularia*, a serious insect pest of litchi. Taiwan Agriculture Quart. 6, 133–143.
Chapman, K.R. (1983) Litchi (*Litchi chinensis* Sonn.), pp. 179–191. In: *Australian Tropical Tree Fruits*, Queensland Government Printer, Brisbane.
Chapman, K.R. (1984a) Tropical fruit cultivars collecting in S.E. Asia and China. Queensland Dep. Primary Ind. p. 123.
Chapman, K.R. (1984b) Sapindaceae, pp. 179–191. In: *Tropical Tree Fruits for Australia*. P.E. Page (ed.). Queensland Dept. of Primary Industries, Queensland, Australia.
Chaturvedi, R.B. and Saxena, G.K. (1965) Studies on the blossom biology of litchi (*Litchi chinensis* Sonn.). Allahabad Farmer 39, 10–13.
Chaturvedi, O.P. and Jha, M.K. (1998) Crop production and economics under (*Litchi chinensis* Sonn.) plantation across 1- to 9-years age series in north Bihar, India. Int. Tree Crops J. 9, 159–168.
Chen, XD., Chen, J.S., Zhang, F.B., and Yang, S.H. (1998) Effectiveness of nitrogen and potash fertilizers on litchi. Guangdong Agri. Sci. 2, 27–29.
Chin, H.F., Hor, Y.L., and Lassim, M.B.M. (1984) Identification of recalcitrant seeds. Seed Sci. Technol. 12, 429–436.
Chin, H.F. and Hor, Y.L. (1989) Excised embryos – a new alternative to seed storage. International Seed Testing Association Congress, Edinburgh.
Chrispeels, M.J., Grossi, M.F., and Higgins, T.J.V. (1998) Genetic engineering with alpha amylase inhibitor seeds resistant to bruchids. Seed Sci. Res. 8, 257–263.
Cull, B.W. (1977) Report on Overseas Study Tour. Queensland Department of Primary Industries, pp. 1–92.
Cull, B.W. and Hams, F.D. (1974) Litchi finds a new home in Queensland. Queensland Agri. J. 100, 597–603.
Cull, B.W. and Paxton, B.F. (1982) Litchi (*Litchi chinensis*). Yearbook of western Australia Nut Tree Crop Association 7, 13–21.
Das, C.S. and Chowdhary, K.R. (1958) Floral biology of litchi (*Litchi chinensis* Sonn.). South Indian Hort. 6, 17–622.
Das, D.K. Prakash, S.M., and Sarin, N.B. (1999) Multiple Shoot induction and plant regeneration in litchi (*Litchi chinensis Sonn*.). Plant cell Rep. 18, 691–695.
De Candolle (1909) *Origin of Cultivated Plants*. Tribner & Co. Ltd., London.
Degani, C., Beiles, A., Bastri, R.E., Goren, M., and Gazit, S. (1995) Identifying lychee cultivars by isozyme analysis. KJ Amer. Soc. Hort. Sci. 120, 307–332.
Ding, X.D., Xin. L.L., Jing, C.X., Xiong, G., Ding, L., Lu, X.D., Chen, X., and Guan, X. (2000) Identifying litchi cultivars and evaluating their genetic relationships by RAPD markers. J. Trop. Subtrop. Bot. 8, 49–54.
Dixon, T., Batten, D., and Mcconchie, C. (2005) Genetic improvement of lychee in Australia. ISHS Acta Horticulture IInd Interntional Symposium on Lychee, Longan, Rambutan and other Sapindaceae plants held at Chiang Mai, Thailand.
Duvenhage, J.A. (1993) Control of post harvest decay and browning of litchi fruit by sodium metabisulphite and lower ph dips. Year book South African Litchi Growers' Association 5, 31–532.
Dwivedi, A.K. and Mitra, S.K. (1995) Genetic diversity of fruit quality traits in litchi (*Litchi Chinensis* Sonn.). Hort. J. 8, 113–118.
Emons, A.M.C. (1994) Somatic embryogenesis: cell biological aspects. Acta Botanica Neer landica 43, 1–14.

FAO (1990) Guidelines for Soil Profile Description, 3rd edition (revised). Food and Agriculture Organization of the United Nations, International Soil Reference Information Centre, Land and Water Development Division. FAO, Rome.

Froneman, J.J. and Oosthuizen, J.H. (1995) ETSG Litchi breeding programme. Yearbook-South African Litchi Growers Association 7, 35–37.

Fu, J.R. Zhang, B. Z., Wang, X.P., Qiao, Y.Z., and Huang, X.L. (1990) Physiological studies on desiccation, wet storage and cryo-preservation of recalcitrant seeds of three fruit species and their excised embryonic axes. Seed Sci. Technol. 18, 743–754.

Fu, L. and Tang, D.Y. (1983) Induction of pollen plants of *Litchi* trees (*Litchi chinensis Sonn*.). Acta Genetica Sinica 10, 369–374.

Goto, Y.B. (1960) Lychee and its processing. Pacific Region Food conference 1, 15–23.

Galan, S.V. (1989) *Litchi Cultivation*. Food and Agriculture Organization of the United Nations, Rome. 136 p.

Gaur, G.S. and Bajpai, P.N. (1978) Some aspects of developmental physiology of the litchi fruits. Indian J. Hort. 35, 173–177.

George, E.F. and Sherrington, P.D. (1984) *Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories*. Exegetics Ltd., Basingstoke, UK.

Gray, D.O. and Fowden, L. (1962) (Methylene cyclopropyl)glycine from Litchi seeds. Biochem J. 82, 853–389.

Groff, G.W. (1921) The Lychee and Longan. Orange Judd company and Canton Christian College, China Sci. Cult. 38, 39–40.

Groff, G. W. (1943) Some ecological factors involved in successful lychee cultivars Fla. State Hort. Soc. Proc. 56, 34–155.

Gupta, D., Bhatia, R., Sharma, N.K., Chandel, J.S., and Sharma, R. (1997) Incidence and management of red rust of litchi in lower hills of Himachal Pradesh. Pest Management in Horticultural Ecosystems 3, 70–74.

Hamilton, R.A and Yee, W. (1970) Lychee cultivars in Hawaii. Proc. Trop. Am. Soc. Hortic. Sci. 14, 7–12.

Hasan, M.A. and Chattopadhyay, P.K. (1993) Fruit quality of litchi under the influence of nitrogen, phosphorus and potassium nutrition. J. Potassium Res. 9, 360–364.

Hayes, W.B. (1957) *Fruit Growing in India*. Kitabistan, Allahabad, pp. 323–332.

Huang, H. and Xu, J. (1983) The developmental patterns of fruit tissues and their correlative relationships in (*Litchi chinensis* Sonn.). Sci. Hort. 19, 355–362.

Huang, H. and Qiu, Y. (1987) Growth correlations and assimilate partitioning in the arillate fruit of (*Litchi chinensis* Sonn.). Aust. J. Plant Physiol. 14, 181–188.

Huang, X., Zeng, L., and Huang, H.B. (2005) Lychee and Longan production in China. ISHS Acta Hort 665: IInd International Symposium on Litchi Longan, Rambutan and other Sapindaceae Plants, held at Thailand.

Jain, S.M. (2005) Major mutation-assisted plant breeding programs supported by FAO/IAEA. Plant Cell Tiss. Org. Cult. 82, 113–123.

Jain, S.M. (2006) Mutation-assisted breeding in ornamental plant improvement. Acta Hort. 714, 85–98.

Jain, S.M. and Maluszynski, M. (2004) Induced mutations and biotechnology on improving crops, pp. 169–202. In: *In Vitro Applications in Crop Improvement: Recent Progress*. A. Mujib, M. Cho, S. Predieri, and S. Banerjee (eds.). IBH-Oxford, India.

Jaiswal, B.P., Jha, A.K., Sah, N.L., and Prasad, U.S. (1982) Characteristics of fruit growth and development in litchi Cultivars. Indian J. Plant Physiol. 25, 411–414.

Jawanda, J.S. and Singh, S. (1977) Fruit yield, ripening time and fruit quality of litchi cultivars grown in Punjab. Punjab Hortic. J. 17, 122–125.

Johnson, G.I. and Sangchote, S. (1994) Control of post harvest diseases of tropical fruits: challenges for the 21st Century. ACIAR Proc. 58, pp. 140–161.

Joubert, A.J. (1970) The Litchi. South Africa Dep. Agric. Tech. Sci. Bull. 389, 1–22.

Kadaman, A. and Slor, E. (1974) Experiments with propagation of the litchi (*Litchi chinensis*) in Isreal. Indian J. Hort. 31, 28–33.

Kane, M.E. (1996) Propagation from preexisting meristems. In: R.N. Trigiano and D.J. Gray (Eds.), *Plant Tissue Culture Concepts and Laboratory Exercises*, CRC Press, Boca Raton.
Kantharajah, A.S., McConchie, C.A., and Dodd, W.A. (1989) The possible contribution of tissue culture to the lychee industry. Proceedings of the Second National Lychee Seminar, Cairns, pp. 59–65.
Kantharajal, A.S., Dodd, W.A., and McConchie, C.A. (1992) *In vitro* embryo culture and induction of multiple shoots in lychee (*Litchi chinensis* Sonn.). Ann. Bot. 70, 153–156.
Kanwar, J.S., Nijjar, G.S., and Rajpoot, M.S. (1972a) Fruit growth studies in litchi (*Litchi chinensis* Sonn.) at Gurdaspur (Punjab). Punjab Hortic. J. 12, 146–151.
Kanwar, J.S., Nijjar, G.S., and Rajpoot, M.S. (1972b) Sun burning and skin cracking in some varieties of litchi(*Litchi chinensis* Sonn.) and the factors affecting them. Indian J. Agric. Sci. 42, 772–775.
Khurshid, S., Ahmad, I., Anjum, M.A. 2004. Genetic diversity in different morphological characteristics of litchi (*Litchi chinensis* Sonn.). Intern. J. Agric. & Biol. 6, 1062–1065.
Koen, T.J., Langenegger, W., and Smart, G. (1981a) Determination of the fertilizer requirements of litchi trees. Inf. Bill. Citrus and Sub Trop. Fruit Res. Inst. 103, 9–11.
Koen, T.J., Langenegger, W., and Smart, G. (1981b) Nitrogen fertilization of litchi trees. Inf. Bull. Citrus Subtrop. Fruit Res. Inst. 117, 1–2.
Koen, T. J. and Smart, G. (1982) Effect of optimal manuring on the production and fruit quality of litchi trees. Information Bulletin of the Citrus and Subtropical Fruit Research Institute 117, 1–2.
Kumar, R. (1977) Performance of elite litchi cultivars under Bihar plateau. The Orissa J. Hort. 25, 18–20.
Kumari-Singh, A. and Prasad, U.S. (1991) Dehydration pattern and viability loss in seed of two cultivars of litchi *(Litchi chinensis* Sonn.). Seed Res. 19, 41–43.
Kumar, M., Gupta, M., Shrivastava, D., Prasad, M., Prasad, U.S., and Sarin, N.B. (2006) Genetic relatedness among Indian litchi accessions (*Litchi chinensis* Sonn.) by RAPD markers. Intl. J. Agri. Res. 1, 390–400.
Kumar, M. (2006) Mass Scale Propagation of (*Litchi chinensis* Sonn.) Through in vitro Technoques. Ph.D. Thesis, Bhagalpur University, Bhagalpur.
Lal, R.L. and Tiwari, J.P. (1996) Effect of nitrogen on yield and quality of litchi (*Litchi Chinensis*). Agric. Sci. Digest Karnal 16, 138–140.
Lal, B.S. and Nirwan, R.S. (1980) Saharanpur Selection a new litchi cultivar. Prog. Hort. 12, 75–77.
Liang J. (1981) The litchi, origin, utilisation and development of its culture. J. Agric. Trinidad Bot. App. 28: 259–270.
Liao, Y.W. and Ma, S.S. (1998) Adventitious embryogenesis of *Litchi chinensis.* J. Chinese Soc. Hortic Sci. 44(1), 29–40.
Lin, C. and Mei M. (2005) Classification of lychee cultivaers with RAPD analysis. ISHS Acta Horticulturae 665: IInd International symposium on Lychee, Longan, Rambutan and other Sapindaceae Plants, Held in Thailand.
Liu, S.Y. (1954) Stuidies on (*Litchi chinensis* Sonn.) Dissertation Abstract 14, 1018–1019.
Loebel, R. (1976) The litchi. Trop. Fruit Bull N.S.W. Dep. Agric 6, 1–12.
Marloth, R.H. (1947) The Litchi in South Africa I.Varieties and Propagation II Cultural Practices and Marketing. Farming South Africa 22, 824–830 and 863–870.
Menzel, C.M. (1985) Propagation of lychee: a review. Sci. Hort. 25, 31–48.
Menzel, C.M. and Simpson, D.R. (1987) Lychee Nutrition: A Review. Sci. Hort. 31, 195–224.
Merkle, S.A. (1995) Strategies for dealing with limitations of somatic embryogenesis in hardwood trees. Plant Tissue Culture and Biotechnol. 1(3), 112–121.
Mishra, K.A. (1982) The vegetative propagation of *persimmon*. Scientia Hort. 17, 125–127.
Mishra, H.N. and Mishra, R.S. (1998) Diallel analysis for variability in bitter gourd (*Momordica charantia*). Ind. J. Agricultural Sci. 68(1), 18–20. (Natl. Agric. Res. Project, Regional Res. Stn., OUAT, Semiliguda, Sunabeda 763 002, India).
Morevil, C. (1973) Some observations and experiments on the litchi. Fruits 28, 637–640.

Mustard, M.J., Liu, S.Y., and Nelson, R.O. (1953) Observations of floral biology and fruit setting in lychee varieties. Proc. Florida State Hort. Soc. 66, 212–220.
Nielson, G. (1985) The use of isozymes as probe to identify and label plant varieties and cultivars. In: *Isozymes :Current Topics in Biological and Medical Research*. M.C. Rattazzi, J.G. Scandilios, and G.S. Whitt (eds). Alan R. Liss, New York, 12, 1–32.
Ochse, J.J., Soule, M.J., Dijkman, M.J., and Wehlburg, C. (1961) *Tropical and subtropical agriculture*. The Macmillan Co., New York. pp1446.
Ouyang, S. and Zheng, X. (1985) T-DNA transfer and tumor formation induced by *Agrobacterium tumefaciens* on *Litchi chinensis. Acta Genetica Sinica* 12, 42–45.
Pandey, R.S. and Yadava, R.P.S. (1970) Pollination of litchi (*Litchi chinensis*) by insects with special reference to honey bees. J. Agric. Res. 9, 103–105.
Pandey, R.M and Sharma, H.C. (1989) The litchi ICAR, New Delhi, India, 1–79.
Paull, R.E. and Chen, N.J. (1987) Effect of storage, temperature and wrapping on quality characteristics of litchi. Fruit. Sci. Hort. 33, 223–236.
Pivovaro, S.Z. (1974) Studies on the floral biology and the influence of growth regulators on fruit set, size and drop of (*Litchi chinensis* Sonn.). M.Sc. Thesis, Hebrew University, Rehovat.
Prasad, U.S. (1977) Physiological studies on ripening of litchi of Bhagalpur Ph.D. Thesis, Bhagalpur University, Bhagalpur.
Prasad J.S. and Prasad, U.S. (2004) Desiccating sensitivity of (*Litchi chinensis* Sonn.) seeds. Indian J. Plant Physoil. (N.S.), 372–377.
Prasad, J.S., Kumar, R., Mishra, M., Singh, A.K., and Prasad, U.S. (1996) Characteristics of litchi seed germination. Hort. Sci. 31, 1187–1189.
Prasad, U.S. (2000) Growth and ripening of litchi fruits. In: *Advances in Plant Reproductive Biology*. A.K. Pandey and T. Dhakal (eds.). Narendra Publishjing House, Delhi, 132–134.
Puchooa, D. (2004) Expression of green fluorescent protein gene in litchi (*Litchi chinensis* Sonn.) tissues. J. Appl. Hort. 6, 11–15.
Rai, P.S. and Bhandary, K.R. (1973) Occurrence of *Xyleborus fornicatus* Elchhof (Coleoptera:Scolytidae) on litchi (*L.chinensis Sonn*.) in India. Curr. Sci. 40, 369.
Ray, P.K. and Sharma, S.B. (1985) Viability of Litchi Chinensis seeds when stored in air and in water. J. Agric. Sci. Camb. 104, 247–248.
Ray, P.K., Mishra, R.A., and Sharma, S.B. (1985) Yield and bearing constancy of some commercial litchi cultivars. Indian J. Hort. 42, 218–222.
Roth, G. (1963) Post harvest decay of litchi fruit (*Litchi chinesis Sonn*.). Technical Commun., Dept. Agric. Tech. Serv., South Africa, 16 pp.
Sarin, N.B. and Prasad, U.S. (2003) *In vitro* regeneration and transformation of litchi (*Litchi chinensis* Sonn.), pp. 211–222. In: *Plant Genetic Engineering, Improvement of Fruits*. P.K. Jaiwal and R.P. Singh (eds.). Vol. 6, Sci. Tech. Publishing LLC, USA.
Sarin, N.B., Prasad, U.S., Kantharaja, A.S., and Jain, S.M (2003) Micropropagation of litchi (*Litchi chinensis* Sonn.), pp. 721–731. In: *Micropropagation of Woody Trees and Fruits*. S.M. Jain and K. Ishii (eds.). Vol. 75, Kluwer Academic publishers, The Nether-Lands.
Sethi, V. (1985) A simple and low cost preservation of litchi juice. Indian Food Packer 39, 42–48.
Sharma, R.C. and Mahajan, B.V.C. (1997) Studies on the fertilizer requirements of litchi (*Litchi chinensis* Sonn.) cv. Calcuttia. Agric. Sci. Digest Karnal 17, 135–138.
Shukla, K.S., Mishra, R.L., Kaul, M.K., and Prasad, A. (1978) Studies on bollen germination of certain fruits. Haryana J. Hort. Sci. 7, 162–164.
Singh, R.D. (1977) Morphological changes during development of litchi fruits (*Litchi chinensis* Sonn.) cultivar. 'Early Large Red' Agriculture and Agro -Industry J. 10, 31–33.
Singh, R.K. and Sarin, P.L. (1957) Studies on the varietal difference in fruit quantity in litchi (*Litchi chinensis* Sonn). Indian J. Hort. 14, 103–107.
Singh, S. and Dhillon, B.S. (1983) Flowering behavior of litchi cultivars under Punjab conditions Indian J. Hort. 40, 14–17.
Storey, W.B., Hamilton, R.A., and Nakasone, H.Y. (1953) Groff – a new variety of lychee. Circular Hawaii Agric. Exp. St. 39, 1–8.

Steiner, J.J. and Greene, S.L. (1996). Proposed use of ecological descriptors and their utility for plant germplasm collections. Crop Sci. 36, 439–451.
Strauss, M.L., Pino, J.A., and Cohen, J.J. (1998) Quantification of diversity in ex situ plant collections. Diversity 16, 30–32.
Subhadrabandhu, S. (1990) *Lychee and Longan Cultivation in Thailand*. Rumthai Publication, Bangkok, Thailand, 40p.
Syamal, M.M. and Mishra, K.A. (1984) Litchi cultivation in India. Farmer & Parliament: 15–16 and 27–8.
Syamal, M.M. and Mishra, K.A. (1989) Litchi cultivation in India Farmer and Parliament 1915–16 and 27–28.
Tao, R. (1955) The Superior lychee. Proc. Florida Lychee Growers' Association 2, 73–74.
Tongpamnak, P., Patanatara, A., Srinivas, P., and Babprasert, C. (2002) Determination of genetic diversity and relationshiops among Thai litchi accessions by RAPD and AFLP markers. Kasetstart J. Nat. Sci. 36, 370–380.
Underhill, P.A., Jin, L., Lin, A.A., Mehdi, S.Q., Jenkins, T., Vollrath, D., Davis, R.W., Cavalli-Sforza, L.L., and Oefner, P.J. (1997) Detection of numerous Y chromosome biallelic polymorphisms by denaturing high-performance liquid chromatography. Genome Res. 7, 996–1005.
Ussuf, K.K., Laxmi, N.H., and Mitra, R. (2001) Proteinase inhibition :Plant derived genes of insecticide protein for developing in sect resistant transgenic plants. Curr. Sci. 80, 847–853.
Villiers, E., De, A., and Mathee, W.A. (1973) The Occurrence of bark borers on litchi and other fruit trees. Citrus Growers Subtrop. Fruit J. 477, 17–19.
Vevai, E.J. (1971) Know your crop, its Pest problems and control of minor tropical fruits. Pesticides 5, 31–54.
Vasil, V. and Vasil, I.K. (1982) The ontogeny of somatic embryos of *Pennisetum americanum (L) K. Schum.* in cultured immature embryos. Bot. Gaz. 143, 454–465.
Watt, B.K. and Merril, A.L (1963) Composition of foods. U.S. Dep. Agric. Hand book No. 8.
Williams, E.G. and Maheswaran, G. (1986) Somatic embryogenesis: factors influencing coordinated behavior of cells as an embryogenic group. Ann. Bot., 57, 443–462.
Winks, C.W., Batten, V. and Burt, J.R. (1983) Australia Sub tropical Horticulture Mission to the People's Republic of China. Common Dep. Primary Ind. Canbera, 75pp.
Wolf, L.M. (1987) In vitro propagation of lychee (*Litchi chinensis* Sonn.). Honours Thesis, University of Queensland, Brisbane, Australia.
Xia, Q., Chen, H.R.Z., and Fu, J.R. (1992a) Effect of desiccation, temperature and other factors on the germination of lychee (*Litchi chinensis* Sonn.) and longan (*Euphoria longan steud*.) seeds. Seed Sci. Technol. 20, 119–127.
Xia, Q., Chen, H.R.Z., and Fu, Z J.R. (1992b) Moist storage of lychee (*Litchi chinensis* Sonn.) and longan (*Euphoria longan steud*.) seeds. Seed Sci. Technol. 20, 269–279.
Yamdagani, R., Balyan, D.S., and Jindal, P.C. (1980) A Note on the effect of nitrogen on litchi (*Litchi chinensis* Sonn.). Haryana J. Hort. Sci. 9, 141–143.
Young, T.W. (1970) Some climatic effects on flowering and fruiting of 'Brewster' lychee trees in Florida. Proc. Florida State Hort. Soc. 83, 362–367.
Yu, C.H. and Chen, A.G. (1998) Embryogenic suspension culture and protoplast isolation in Litchee. Chinese J. Trop. Crops 19, 16–20.
Yu, C., Chen, Z., Lu, L., and Lin, J. (2000) Somatic embryogenesis and pant regeneration from litchi protoplasts isolated from embryogenic suspensions. Plant Cell Tiss. Org. Cult. 61, 51–58.
Zhou, L.N., Kuang, Z.S., Ma, X.J., Chen, J.Q., and Cao, J. (1996) The Study of factors affecting somatic embryogebnesis in young embryo culture of *Litchi chinensis* . J. Agric. Biotech. 4, 161–165.