Chapter – I

Introduction

Banana and plantains (*Musa* spp. L.) are the important staple and cash crops that grow in the humid regions of Africa, America and Asia (Robinson, 1996). ‘Banana’ refers to those cultivars that are eaten raw when ripe, like the dessert bananas, while ‘plantain’ is those eaten green, or ripe, but cooked or fried. In other words green banana which becomes palatable after cooking, are popularly referred as plantain while the fresh fruit consumed after ripening, is referred as dessert banana. Worldwide annual production is estimated to exceed 100 million tones, which are distributed between Africa (40%), Asia (30%), Latin America and the Caribbean (30%). India is the largest producer of banana with 17.0 million tones from an area of 6.8 lakh hectares (FAO, 2005).

Banana and plantains are the source of staple food in the equatorial belt of Africa, where more than 70 million people derive in excess of 25% of their daily calorie intake from plantains (Robinson, 1996). They are known as wonder berry, forming staple food for millions of people across the globe and provide a more balanced diet than any other fruit or vegetable. Bananas and plantains are sources of dietary carbohydrate, vitamin C and number of minerals (Nweke *et al.*, 1988). They are also a good source of major elements such as potassium, magnesium, phosphorous, calcium, iron and vitamins like A, B6 and C (Marriott and Lancaster, 1983).

Banana is referred as “Kalpatharu”, a plant of all virtues, with each and every part of the plant being used for various purposes. The importance of banana as a food-fruit crop can hardly be exaggerated adding to its multifaceted uses as food, fibre, fuel and therapeutic values. Leaf is the most popular hygienic bio-plate for dining, male flower is much preferred as vegetable and for making pickles, and stem is also a vegetable in demand with lot of therapeutic uses. Banana is also a good fiber yielding plant and its corm is mostly exploited as animal feed, as composite mixture with others (Uma *et al.*, 2005). Bananas and plantains are therefore the important components of
food security in the tropical world and they provide income to the farming community through local and international trade.

1.1 Taxonomy

Botanically, banana and plantains are perennial giant monocotyledonous herbs, belonging to the order Zingiberales (Scitaminae) and family Musaceae. The Musaceae consists of two genera Musa L. and Ensete L. The genus Ensete is monocarpic, with a basic chromosome number 9 and bears no edible fruits. The genus Musa is more widely distributed and comprises of four sections (Eumusa, Rhodochlalymis, Australimusa and Callimusa) with basic chromosome number between 9 and 14. The edible fruit bearing species are contained in the sections Eumusa and Australimusa. Simmond and Weatherup (1990) recently revised the morphological data, dividing Eumusa into two sub-sections: Eumusa (1) and Eumusa (2). The sections Rhodochalymus and Callimusa comprises a number of non-domesticated species, which are more of ornamental importance (Cheesman, 1947; Simmonds, 1966; Swennen and Rosales, 1994). Recently one more section has been added i.e. Ingentimusa (Simmonds and Weatherup, 1990). This detailed taxonomic classification done by Cheesman, (1947) is the revised form of the classification done by Baker (1893). It is based on chromosome number, pseudostem stature, inflorescence characters and seed morphology.

1.2 Origin, evolution and diversity

Banana and plantains are believed to have originated from South and South East Asian countries along with Pacific Islands especially Papua New Guinea. India is considered as one of the major centres of origin for Musa and origin of wild bananas stretches from India upto Pacific Islands including Papua New Guinea, Micronesia, Melanesia, Samoa etc. (De Langhe et al., 2005). Besides India, African countries are assumed to be the cradle of banana diversity especially plantains where both cultivated and wild forms are found. The humid lowlands of West and Central Africa are the centre of diversification for plantains, while the highlands of East Africa are centres of diversification for cooking and beer bananas (Swennen and Vuylsteke, 1991).

The new cultivars evolved in a new environment faced fertility barriers, resulting in meiotic abnormality and mutations creating parthenocarpic cultivars. This natural hybridization and natural selection under varied ecological climates continued
resulting in *Musa* variability until the human intervention for selection of only desired cultivars (Valmayor, 2001). Both bananas and plantain are derived from intra and interspecific crosses between two diploid wild species *Musa acuminata* Colla (genome A) and *Musa balbisiana* Colla (genome B), which belongs to the same section *Eumusa* (Simmonds, 1995). This is the biggest section in the genus and the most geographically widespread, with species being found throughout Southeast Asia from India to the Pacific Islands (Horry *et al.*, 1997). The different *Musa* cultivars and groups are designated by different combination of ‘A’ and ‘B’ to indicate the relative contributions of *M. acuminata* and *M. balbisiana* respectively, to their genomes.

Both *M. acuminata* and *M. balbisiana* serves as progenitor for majority of the cultivated bananas, which may be diploid (2n=22), triploid (2n=33) or tetraploid (2n=44). Most cultivated *Musa* are triploids (2n=3x=33) with characteristic genome constitutions (Lebot *et al.*, 1993), while diploids and tetraploids are lesser in number. Simmonds and Shepherd (1955), proposed the following genomic classes for edible *Musa AA, AB, AAA, AAB, ABB, AAAA and ABBB*. These genomic groupings are almost exclusively used for the classification of edible *Musa* (Turner, 1994).

Within *Eumusa* the two species *M. acuminata* and *M. balbisiana* are widely distributed. *M. acuminata* though originated either in Malaysia (Simmonds, 1962) or Indonesia (Nasution, 1991 and Horry *et al.*, 1997), they are predominantly spread in Pacific Islands, Indonesia, Malaysia, Philippines, Myanmar and India. On the other hand, original populations of *M. balbisiana* are located in India and are widely distributed from there to Philippines and New Guinea (Simmonds, 1962). Nevertheless, few different accessions were known in the collections, and weak relative diversity has been observed (Horry, 1989 and Carreel *et al.*, 1994).

The first step in the evolution of edible bananas was the development of parthenocarpy and seed sterility in *M. acuminata*. Parthenocarpy is the ability of the fruits to grow and develop into full and edible parenchymatous pulp without pollination. Seed sterility is due to cytogenetic factors and is very important, because banana seeds are stony and unpleasant to encounter. Edibility is, therefore, parthenocarpy plus seed sterility and human selection for these traits (Simmonds, 1987). The edible cultivars are in decreasing order of numerical importance, triploid
AAB and ABB, tetraploid AAAB and AABB, triploids AAA and tetraploid AAAA and ABBB. Triploids are predominant because they are superior to diploids in terms of vegetative vigour and yield. Tetraploids are as vigorous and productive as triploids and are also useful in breeding programmes (Simmonds, 1987).

Until recently only *M. acuminata* (A-genome) and *M. balbisiana* (B-genome) were thought to be the progenitors of present day bananas (Cheesman, 1948). But identification of *M. schizocarpa* and *M. textiles* represented by S and T genomes in cultivated and wild types, has added a new dimension to the evolutionary theory. A Philippine clone “Butuhan” (BT) is one such example which was considered to be formed by the hybridization between *M. balbisiana* (B) of section *Eumusa* and *M. textiles* (T genome) of section *Australimusa* (Carreel, 1994).

1.3 Indian commercial cultivars of banana

1.3.1 Grand Naine (AAA)

It is a popular commercial cultivar grown extensively for table and processing purpose in the states of Maharashtra, Gujarat, Bihar and West Bengal. It is also popular in Tamil Nadu, Karnataka and Andhra Pradesh. Grand Naine is the leading commercial variety of Cavendish group. The bunch size, the fruit length and size is quite good though the keeping quality is poor. The average bunch weight with 6-7 hands and with about 13 fruits per hand is about 15-25 kg. The selection yields bunch weighing 60-70 kg. It is highly susceptible to Sigatoka leaf spot disease in humid tropics restricting its commercial cultivation.

1.3.2 Robusta (AAA)

It is a semi-tall variety, grown mostly in Tamil Nadu and some parts of Karnataka for table purpose. It is a high yielding variety and produces bunch of large size with well developed fruits. Dark green fruits turn bright yellow depending on ripening conditions. Fruit is very sweet with a good aroma. Bunch weighs about 25-30 kg. Fruit has a poor keeping quality leading to a quick breakdown of pulp after ripening, hence not suited for long distance transportation. Robusta is highly susceptible to Sigatoka leaf spot disease in humid tropics.
1.3.3  Rasthali (Silk AAB)

It is a medium tall variety commercially grown in Tamil Nadu, Andhra Pradesh, Kerala, Karnataka and Bihar. Its unique fruit quality has made Rasthali popular and a highly prized cultivar for table purpose. Fruits are yellowish green throughout their development, but turn pale yellow to golden yellow after ripening. Fruit is very tasty with a good aroma. Longer crop duration, severe susceptibility to *Fusarium* wilt, requirement of bunch cover to protect fruits from sun cracking and formation of hard lumps in fruits make the crop production more expensive.

1.3.4  Poovan (Mysore AAB)

It is a leading commercial cultivar grown throughout the country specifically in Kerala, Tamil Nadu, Andhra Pradesh and North Eastern Region. Tamil Nadu is the leading producer of Poovan cultivar. Fruit is slightly acidic, firm and has typical sour-sweet aroma. Fruits turn to attractive golden yellow on ripening. Medium sized bunch, closely packed fruits, good keeping quality and resistant to fruit cracking are its plus points. But it is highly susceptible to Banana Bract Mosaic Viral (BBMV) disease and Banana Streak Virus, (BSV), which cause considerable reduction in yield.

1.3.5  Nendran (AAB)

It is a popular variety in Kerala where it is relished as a dessert fruit as well as for processing. Commercial cultivation of Nendran has picked up rapidly in Tamil Nadu in the recent past. Bunch has 5-6 hands weighing about 12-15 kg. Fruits have a distinct neck with thick green skin turning buff yellow on ripening. Fruits remain starchy even on ripening. Nendran is highly susceptible to Banana Bract Mosaic Virus (BBMV), nematodes and borers.

1.3.6  Ney Poovan (AB)

Ney Poovan is the choicest diploid cultivar, which is under commercial cultivation on a large scale especially in Karnataka and Tamil Nadu. Ney Poovan is a slender plant bearing bunches of 15-30 kg after 12-14 months. Dark green fruits turn golden yellow with a very good keeping quality. Fruit is highly fragrant, tasty, powdery and firm. Ney Poovan is tolerant to leaf spot but susceptible to *Fusarium* wilt and banana bract mosaic virus.
1.3.7 Monthan (ABB)

It is a widely cultivated variety for culinary purpose. Monthan is a fairly tall and robust plant bearing bunches of 18-20 kg after 12 months. Fruits are bold, stocky, knobbed and pale green in colour. The skin is usually green. The new prolific 'Monthan' type clones of economic value namely 'Kanchi Vazhai' and 'Chakkia' are recently becoming popular in Tamil Nadu. It has many desirable qualities like immunity to Banana Bunchy Top Virus (BBTV) diseases, salt tolerance and normal bunch mass even under marginal condition, but it is highly susceptible to Fusarium wilt disease.

1.3.8 Karpooravalli (ABB)

It is a popular variety grown for table purpose in medium rich soils. Its commercial cultivation is spread over in Central and Southern districts of Tamil Nadu and Kerala. It is also the sweetest among Indian bananas. Fruits are ashy coated with golden yellow, they are sweet with good keeping quality. Karpuravalli is highly susceptible to wilt disease, tolerant to leaf spot disease and well suited for drought, salt affected areas and for low input conditions.

1.4 Production constraints in Banana

The yield of banana and plantain has been declining due to increasing pest and disease pressure (Vuylsteke et al., 1993). One of the major constraints is virulent forms of Sigatoka leaf spot diseases both black and yellow (Mycosphaerella fijiensis and M. musicola). Fusarium wilt or Panama disease (Fusarium oxysporum), banana weevil (Cosmopolites sordidus) infestation and compels of plant parasitic nematodes like (Radopholus similis, Pratylenchus coffeae, Meloidogyne incognita, Helicotylenchus multicinctus etc.) are also other important devastating factors in different regions. Cucumber mosaic virus (CMV), banana bunchy top virus (BBTV) and banana streak virus (BSV) diseases have also been reported in several parts of the world.

1.5 Conventional method of propagation

Banana is propagated by suckers that arise from the underground rhizome. It is mandatory that the planting material should be free from insect pests and diseases so as to yield healthy and productive plants. However, propagating bananas by this method has some disadvantages like the bulkiness of planting materials and their difficulty in
transportation. In this method, proper identification of clones in younger stages may not be possible and rapid multiplication of new hybrid cultivars becomes difficult as the process is very slow and the multiplication rate of suckers is at 5-20 per year depending on the clone and the agroclimatic conditions and cultural methods followed (Shanmugavelu et al., 2000).

Conventional plant breeding and selection is of course, the common vehicle whereby improved cultivars are routinely produced. While this is acceptable for those plants that are fertile and produce viable embryos, species that do not do so pose special problem. In banana, intractable fertilization barriers such as moderate to high levels of female sterility and triploidy, slow propagation and complex pattern of gene inheritance make genetic improvement of parthenocarpic Musa clones slow and technically difficult. Owing to its plant-based constraints, banana breeding has also proven to be difficult, time consuming and very expensive. Moreover, hybrid plant production in the most common triploid clones is further complicated by low seed set and germination (Shepherd et al., 1996).

1.6. Importance of non conventional methods

Genetic transformation technique seems to be an useful method to produce novel breeding materials because this can make a good use of isolated genes from a variety of plants species. The application of genetic engineering, transformation, molecular biology and other advanced techniques in the field of biology has allowed plant breeders to identify, select and transfer genes from one plant to another through a process called genetic transformation. This process is far faster, more efficient and more precise than conventional plant breeding techniques. It has also allowed the exchange of genes between organisms that cannot be crossed sexually.

The need for genetic engineering and the knowledge of biosystematic relation, and possibility to overcome crossability barriers can be greatly improved by in vitro techniques. Haploidization, protoplast fusion, gene transfer and exploitation of somoclonal variation are some examples of in vitro culture techniques of potential importance for crop improvement. However, there is no universally applicable method of culture, regeneration and transformation for all species, as tissues from different genotypes will differ in their response to culture. A procedure to produce shoots
through regeneration from one cultivar may be very different from that of another cultivar within the same species. Therefore culture and regeneration protocols must be modified appropriately for culture of each species (Walden and Wingender, 1995).

1.6.1. **Importance of in vitro system in improvement of *Musa***

Tissue culture technique and genetic engineering are of special value for banana. These techniques have been pivotal in *Musa* improvement at many research stations (Vuylsteke *et al.*, 1993). It has unique advantage of rapid multiplication, uniformity of planting materials, availability of more number of plants in short time, disease-free and also possibility of non-seasonal production of plants over other propagation methods. These *in vitro* techniques can also potentially overcome some of the factors limiting traditional approaches to banana and plantain improvement. These techniques also enable plants to be regenerated from normal and genetically modified cells and tissues in an efficient way under sterile conditions.

In addition, there are several reports which proves that *in vitro* banana plants are superior to the conventional suckers due to their vigorous growth (Daniells, 1988), precocity and higher yields (Drew and Smith, 1990). Moreover, *in vitro* system forms the basis for the successful programme of plant genetic engineering. Plant genetic engineering requires the mastery of a regeneration process by means of organogenesis or somatic embryogenesis, preferably of unicellular origin to avoid the problem of chimeras (Grapin *et al.*, 1996).

1.6.2. **Somatic embryogenesis**

Somatic embryogenesis in *Musa*, using cell suspension has been the subject of research since 1960s (Krikorian and Scott 1995). Somatic embryogenesis is the formation of an embryo from a cell other than a gamete or the direct product of gametic fusion (Merkle *et al.*, 1995). Somatic embryogenesis technique in the genus *Musa* is aimed at two main objectives-the development of high performance micropropagation and regeneration system useful for genetic improvement. Embryogenesis is considered as a model for testing the totipotency of crop tissues. It also provides an ideal system for the investigation of the whole process of differentiation of plants as well as the mechanisms of expression of totipotency in plants.
In the starting phase of banana genetic manipulation, research groups have focused both on the use of meristems (May et al., 1995) and embryogenic cells or protoplasts (Sagi et al., 1995) of banana as basic material for genetic manipulation. Generation of chimaeric transformants hamper the wide application of the techniques used. On the other hand, maximum works in genetic transformation, even when using Agrobacterium-mediated transformation (Perez Hernandez et al., 1998, 1999; Moy et al., 1999), are currently relying on embryogenic cells/tissues. At present, somatic embryogenesis in banana is carried out by making use of vegetative tissues such as rhizome fragments and leaf bases (Novak et al., 1989, Ganapathi and Higgs 1999), proliferating meristem cultures (Dhed’a et al., 1991; Dhed’a 1992; Schoofs 1997; Schoofs et al., 1998) and immature male and female flowers (Escalant and Teisson, 1994; Grapin et al., 1998). Although embryogenic cell suspensions were obtained and plants were regenerated from them by these groups (Dhed’a et al., 1991; Schoofs et al., 1998; Grapin et al., 1996; 1998; Cote et al., 1996), it would be an over statement to say that the production of embryogenic cell suspensions from meristem or immature flowers would be routine and free from problems.

1.6.3. Somaclonal variation in Musa

To regenerate and multiply the existing clone, many micropropagation protocols are developed and a large number of plantlets are produced respectively (Bhagyalakshmi et al., 1995; Venkatachalam et al., 2007). But in banana, the genetic fidelity of regenerated plants is often questioned because there are frequent reports on the occurrence of somaclonal variations not only in regenerated plants but also in micropropagated ones (Smith 1988; Damsco et al., 1996; Martin et al., 2006). Such kind of variations is known as somoclonal variations.

Larkin and Scowcroft (1981) coined the term somaclonal variation to describe the occurrence of genetic variants derived from in vitro procedures. Factors such as explant source, time of culture, time of subculture, number of subcultures, phytohormones, genotype, media composition, the level of ploidy and genetic mosaicism are capable of inducing in vitro variability (Silvarolla, 1992).
1.6.3.1. Identification of genetic variation

The major hindrance of somaclonal variation is the identification of genetic variations within the regenerated plants and their stability in succeeding generations. Genetic stability and maintenance of the variant germplasm are mandatory for crop improvement program. Tissue culture induced variations can be determined at the morphological, cytological, biochemical, and molecular levels with several techniques. Although the morphological traits can be used reliably to differentiate off types, mutants, specific clones. But the discriminating ability of these techniques weaken at *in vitro* level. Molecular markers suitable for generating DNA profiles have proved to be an effective tool in assessing the genetic stability of regenerated plants. These markers are not influenced by environmental factors and generate reliable and reproducible results. Molecular markers are widely used to detect and characterize somaclonal variation at the genetic level (Ford-Lloyd *et al.*, 1992; Barrett *et al.*, 1997). A number of such markers have been successfully used in *Musa*. They are:

- Anthocyanin polymorphisms.
- Isozyme Analysis.
- Random Amplified Polymorphic DNA (RAPD) markers.
- Restriction Fragment Length Polymorphism (RFLP).
- Short Tandem Repeats (STRs).
- Simple Sequence Repeats (SSR).
- Inter Simple Sequence Repeats (ISSR)
- Allele specific Amplified Fragment Length Polymorphism (AS-AFLP).

1.6.3.2. Use of microsatellite marker

The large number of alleles and high level of variability among closely related organisms made PCR amplified microsatellites, the marker system of choice for a wide variety of applications. Microsatellites are short nucleotide tandem repeats of a motif, usually one to six bases. They are present in bacterial, fungal, plant, animal and human genomes, and are often referred to simple sequence repeats (SSR). Microsatellites are easy to amplify and are highly abundant and evenly distributed throughout genome (Weising *et al.*, 2005), which makes the method highly polymorphic and specific (Bornet and Branchard 2001). SSR are used for plant breeding, conservation biology and population genetics as forensics, paternity analysis and gene mapping (Coates and
The methods require little amount of DNA, which does not have to be of high quality. Similarly, Inter simple sequence repeat (ISSR) is a PCR based method, which involves amplification of DNA segments present at an amplifiable distance between two identical microsatellite repeat regions oriented in opposite direction. The technique uses microsatellites, usually 16–25 base pair long primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter-SSR sequences of different sizes. This technique is simple, quick, and efficient. The primers are long resulting in high stringency and hence reproducibility. These primers can be successfully used to study the variations among the population of plantlets produced through different culture techniques.

1.7. Objectives

    Based on the aforesaid facts, the following objectives were designed to:

    Ø Screen embryogenic capacity of Indian commercial cultivars of bananas.
    Ø Study the factors controlling the phenomenon of somatic embryogenesis and embryogenic cell suspension in commercial cultivars of bananas.
    Ø Develop an efficient regeneration and germination system for *in vitro* propagation and genetic improvement of bananas.
    Ø Assess the genetic fidelity of suspension derived plants using molecular markers and morphotaxanomical evaluation in field condition.