Chapter 7
Plantain (*Musa paradisiaca* L.) Genetic Improvement and Germplasm Management with Emphasis on Cross River State in Nigeria

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Abstract In recent years, there has been an increase in demand for plantain as a major staple with enormous potential for domestic and global trade. It provides a vital source of income for many developing countries and increased awareness exists about its nutritional value and nutraceutical properties. However, knowledge regarding the scope of genetic and phenotypic diversity among most commercial plantain cultivars has received less attention. Achievements have been made in the genetic improvements of plantain through in vitro culture techniques, cryopreservation, induced mutation breeding, haploid production and production of virus-free plantain. In addition, estimation of cultivar variability based on molecular markers revealed wide genetic diversity useful for selecting elite genetic resources. Clustering based on scores of standard phenotypic traits delineate plantains into distinct groups, with one of these presenting a dichotomization event that results in both a double and triple bunching phenotype at fruiting. The best agronomic practices adopted for high yield of plantain are also evaluated. Findings suggest the presence of significant variability that symbolizes an excellent opportunity to bring about genetic improvement, management and conservation of plantain germplasm through selection of high-yielding cultivars exhibiting unique traits. This chapter highlights research progress relevant to these aspects.

Keywords Genetic diversity · Inflorescence · Molecular breeding · Micropropagation · Microsatellite markers · Phenotypic plasticity · Plantain

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7.1 Introduction

Plantain (*Musa* spp. AAB genome) is one of the perennial giant herbaceous plants in the genus *Musa*. Plantain is one of the most useful food crops in Africa, where it has been widely utilized and is very prominent ingredient in local delicacies and as a major driver of local agrarian economies (Crouch et al. 1998). The crop remains one of the most important dietary energy providers in the wet humid and subhumid ecological climates of the globe, where it is farmed and provides essential nutrition for more than half a billion people (Ogazi 1996). The fruits (fingers) are of extraordinary significance, providing an important and cheap source of carbohydrates, vitamins, and several essential minerals including potassium, sodium, phosphorus and iron. Presently, Sub-Saharan Africa produces more plantain than the rest of the world combined (FAOSTAT 2018). Presently, in many parts of the African continent, plantain and certain other crops such as rice and manioc constitute all year and/or seasonal staples crops. Plantain also serves as an ingredient in many community cottage industries for the manufacture of beverages and local brewed products (Ssebuliba et al. 2000), chemicals and fermented liquor, which are very nutritious due to their high content of B-complex vitamins and high population of fungal yeast in the brew. Plantain also finds wide utilization in the area of excellent flour production in terms of quality by using the peeled unripe fruit and dried pulp. The flour is a very good source of carbohydrate and is thus prescribed for use by diabetics as it has proven to have higher biological value than dietary energy sources obtained from other food crops (Vuylsteke et al. 1996).

Thus far, progress in plantain production in Africa has depended to a large extent on the ability to select high-yielding cultivars from a segregating population (IITA 2007). And given that the estimation of genetic diversity in a crop species is a prerequisite for its improvement, there is an encouraging possibility that different plantain cultivars can be improved once genetic variability has been ascertained using appropriate selection indices. However, the expected genetic response to selection is determined by heritability and the variability of the traits for which the crop is selected for cultivation are to be made relative to the farming systems prevalent in a given socioeconomic environment. There is no doubt that an accurate knowledge of genetic diversity and relationships among plantain collections in any preserved germplasm is essential and important for the establishment, management and guarantee of long-term success of plantain improvement programs through breeding (Dzomeku et al. 2007).

Plantain cultivars show considerable variations for many horticultural traits such that different criteria including pedigree records, morphological traits and DNA marker technology (Weising et al. 2005) have been used in the past to estimate genetic diversity prevalent within the species.

Like with banana, different plantain-producing areas currently suffer from newly emerging pests and diseases and rapidly changing environmental conditions. In the absence of locally-adapted resistant varieties and a general lack of characterized

germplasm that could be used as potential parents for breeding purposes, farmers need to extensively use pesticides, which threaten the sustainability of not only the crop but also the environment (Pennisi 2010).

There is therefore an urgent need for the selection of cultivars with significant variability of genetically-improved characters with more improved disease resistance, enhanced yield and superior potential to adapt to a robust area with optimum growing conditions. In consideration of the fact that an understanding of the variability among different plantain varieties is desirable for setting up of an efficient strategy for breeding improved cultivars and support the choice of parents that can be used for regeneration, a very robust appreciation of the genetic and phenotypic diversity of available resources is of paramount importance (Jarret and Gawel 1998). Consequently, this chapter is designed to provide details on the diversity available, based on information generated through molecular fingerprinting and variations in morphological and yield-related traits within a large plantain germplasm collection, as a foundation for selection and conservation of genetically-superior cultivars that can be used for further research into the breeding and improvement of this important staple crop (Ogazi 1996; Swennen 1997).

The pool of genetic variation within a parthenocarpic population of plantain is the basis for selection and improvement of plantain germplasm. In recent years, there has been increasing awareness of a holistic approach in agricultural biodiversity conservation for sustainable utilization and development for food security and income generation (Simmonds and Shepherd 1995).

Hence, in the conservation of plantain (*Musa paradisiaca* L.) genetic diversity (germplasm) is indispensable for sustainable agriculture and for the well-being of present and future generations. Thus, understanding the distribution and scope of biodiversity in plantain germplasm availability in Cross River State, Nigeria, would help in the proper conservation and sustainable use, since changes in genetic variability among plantain populations largely depend on time and space. Moreover, the scope and distribution of genetic diversity in the plantain germplasm relies greatly on its evolutionary pathways, breeding systems, ecological and geographical factors, past bottlenecks as well as biotic and other abiotic factors (Brisibe and Ekanem 2019).

This chapter presents an overall view of the historical development, origin and distribution, taxonomic classification, germplasm diversity, genetic, species and ecological diversities of plantain, sustainable agronomic practices, characterization and conservation, traditional and modern cultivation practices for elite plantains cultivated in Cross River state, Nigeria (Fig. 7.1). It also presents recent developments on biotechnology and molecular phylogeny and their application for the crop improvement in association with conventional plantain breeding methods along with utilization.

Fig. 7.1 Map of Cross River State, Nigeria, showing major areas of plantain production. Pink (low production area), Yellow (high production area), Sky blue (average production area). (Source: Fadama III project, Cross River State, Nigeria, 2015)

7.2 Multiplication Techniques Adopted in Cross River State

7.2.1 Split Corm

This is normally obtained from a ball-earth corm that is usually split into several units weighing about 50 g each. The unit should have eyes or buds to facilitate sprouting. Each unit should be treated with fungicides such as Captan or Furadan against infections before spreading in the sun to harden the cut surface. The units are then planted in a nursery for 7–8 weeks for sprouting. The sprouted seedlings are the peepers which are then transplanted at a spacing of 15 × 15 cm into the field (Fogain et al. 1998; Speijer 1959; Wilson 1983).

7.2.2 Bakers Technique

This is also called the stripping technique and involves the stripping of the outer leaves to the base rhizome, which stimulates the growth of auxiliary buds to form suckers. The suckers are removed as soon as they sprout (peepers) and cultured in the nursery (Gerda 1990).

7.2.3 Hamilton Technique

This is carried out on a mature plantain of about 6–12 months of age that has not produced fruits. The pseudostem is cut above the corm and the apical meristem is destroyed, which stimulates and promotes the growth of adventitious buds. Peepers are then removed and cultured in the nursery (Dzomeku et al. 2007).

7.2.4 Use of Peep and Sword Ratoon/Follower Crops Suckers

Most traditional farmers use healthy corms obtained from ratoons and follower crops as planting materials or parent stock. This is mostly practiced by rural communities on small and large plantation farms. This practice has the advantage of sporadic spreading and development of plantains from ratoons and follower peepers and sword suckers which develop within a very short time from the corm. In most cases, the farmers remove the peep and sword suckers to another planting hole using appropriate spacing distance (Fogain et al. 1998).

7.2.5 Traditional Breeding Limitations

The traditional breeding method has several limitations including the inability to check and improve the genetic makeup of the cultivars. The genetic characteristics of the cultivars are not ascertained by farmers before planting, leading to inbreeding depression. Associated diseases such as black sigatoka disease and bunchy top diseases are simply transmitted from one generation to another. There is continuous declining yield and outputs due to low genetic makeup and poor breeding/management practices (Gill 1998).

7.3 Biotechnological Improvement Efforts for Plantain Germplasm

Conventional efforts to propagate, conserve and breed cultivated *Musa* are fraught with obstacles such as low reproductive fertility, slow rate of vegetative propagation and long growth cycle. Interestingly, tissue culture and molecular genetic methods are increasingly being used as enabling techniques for handling and improvement of *Musa* germplasm worldwide. In some research institutes such as the International institute for Tropical Agriculture, Ibadan, Nigeria, shoot-tip culture is routinely used for rapid propagation, safe international exchange and germplasm conservation (Vuylsteke et al. 1996).

7.3.1 Micropropagation

This technique has been crucial for the rapid supply of large numbers of female and male plantain plants for crossing and for the continual supply of promising new hybrids for field evaluation trials. Over 300 new *Musa* accessions have been introduced through shoot-tip culture during the last decade (Vuylsteke 2000).

Plant tissue culture gives the opportunity to grow plant material in artificial conditions with advantages such as: possibility for sanitation (culture in sterile conditions, pathogen cleaning); rescue and conservation (continuous availability, space saving, cost-effectiveness, cryopreservation); micrografting and micropropagation (mass multiplication: bioreactor, cell suspension, polyshoot development); germplasm exchange; open access for more research (genetic transformation, somatic embryogenesis, haploid production, embryo rescue, molecular, pathological/virological investigations) in the production of plantain and other *Musa* species (IITA 2018).

Micropropagation using shoot tip culture reveals that plantain is very responsive to in vitro plant tissue culture meristem regrowth in 4–6 weeks, followed by shoot development proliferation to subculture of fully developed plantlets. Micropropagation of plantain multiplication using semi-solid culture medium reveals that plantain proliferation medium shows an average multiplication rate of 4:1 in semi-solid culture medium components using 4:1 MS basal medium (Murashige and Skoog 1962) supplemented with 4.43 mg/L myo-inositol, 100 mg/L sugar, 30 ml/L indolacetic acid (IAA), 0.18 mg/L benzylamino purine (BAP) and 4.5 mg/L ascorbic acid in 10 mg/L agar (IITA 2018).

7.3.2 Micropropagation of Plantain Using Temporary Immersion System (TIS) Bioreactor

Micropropagation and multiplication of plantain using the temporary immersion system (TIS) bioreactor Rocket kit shaker and Southern sun bioreactor air-lift are instruments used for the micropropagation of plantain plantlets. Micropropagation

of plantain multiplication using the TIS bioreactor showed that the average multiplication rate using TIS was very high. The hardening and acclimatization process revealed more than 95% success of micropropagation using this protocol (IITA 2018).

7.3.3 Somatic Embryogenesis in Musa

In plantain, like any angiosperm, embryo production by using somatic cells shows special attributes of plasticity development in the plant cells. Somatic embryogenesis (SE) induction with somatic embryos using embryogenic cell suspension (ECS) cultures is an authentic achievement protocol for enhancing mass-propagation and production of plantain because of the high percentage of regeneration, and remains a tool to reckon with in cellular proliferation in traditional breeding of the crop. Mechanisms and laboratory approaches used for somatic embryogenesis are basic for many *Musa* species like those having AA and/or BB genomes, wild dessert (AA, AB, AAA), cooking banana (ABB) and cultivated plantain (AAB) using various forms of the explants. Although, in most instances, the procedures are confined to low embryo sprouting and plant establishment rates. Hence, the need for a conscious attempt to understand the biochemical, physiological and genetic procedures underlying plantain zygotic and somatic embryo development, so as to develop a reliable somatic embryogenesis methodology with a high percentage of embryo sprouting, establishment and plant development (Vuylsteke 2000).

An efficient and simple procedure has been developed for explant development using an in vitro somatic embryo protocol in *Musa* spp. In vitro propagation, using the somatic embryogenesis approach, has been exploited to obtain identical planting stock in *Musa* spp. In a current study, somatic embryogenic plants were achieved in selected *Musa* genotypes with immature male flower bud explants. Evaluation carried out using two stages of somatic embryogenesis, including induction and maturation of somatic embryos, revealed that embryogenic calli desiccated for up to 2 h at 25 ± 1 °C resulted in higher frequencies of embryonic induction and maturation, compared to nondesiccated embryos. Regenerated explants are hardened and the genetic fidelity of the plantlets confirmed using sequence-related amplified polymorphism (SRAP) molecular markers. Furthermore, it was observed that plantain plants derived from somatic embryogenesis showed normal morphotypes, the same as phenotypes derivable from a parent plant. The establishment of follower plants from male flower bud was achieved in 6–7 months, which is a relatively good period of time for plantain genotypes when compared to related studies involving different accessions of same plant. The procedure adopted can be very useful for large-scale micropropagation investigations for important commercial species of plantain (Jain 2007).

Somatic embryogenesis signifies a process in which bipolar appendages, similar to a zygotic embryo, develop from a nonzygotic cell without vascular connections to the original tissue. SE is an important system where the multiplication of plantain

plantlets can be carried out speedily. During the development process, somatic embryogenesis undergoes four consecutive stages: (a) somatic embryogenesis induction in the plantlets, (b) formation of somatic embryo, (c) somatic embryo maturation and (d) germination of somatic embryos and their conversion into viable explants (López et al. 2013). Somatic embryogenesis thus remains an ideal approach for the development of procedures to achieve rapid multiplication of plantlets, new genotypes and synthetic seed production. The selection protocol using an in vitro approach utilizes different living and non-living components and their effects to study genetic manipulation. The best established explants are obtained from the proliferating meristems and male bud inflorescences. This allows for the development of embryonic cell suspension (ECS), outright formation of somatic embryos, and the subsequent development into plantlets that are further exposed for field establishment and evaluation. The procedure is based on the use of explant shoot apices derived from the multiplication of the axillary buds in an ancymidol solution (0.2–0.4 mg/L) and depends on the plant genotype used. It also depends on the somatic embryos potential to achieve maximum development in semisolid and liquid culture media. The use of shoot apices of axillary buds to induce embryonic cell suspensions in AAB group plantain cultivars prompted the need to scale up propagation using somatic embryogenesis and temporary immersion system (biofactory) that are being used as an alternative for propagation of good clones (IITA 2018) and its application in the plantain genetic improvement program by mutations (Jain 2010).

7.3.4 Embryo Culture and Rescue Techniques

These techniques are applied to increase the germination rate of true seeds from crosses, with more than 10,000 seeds cultured at once in the laboratory each year.

Unlike suckers, the adoption of explants developed through tissue culture (TC plantlets) show several merits. Tissue culture developed plants tend to be cheaper and show easily transported and propagated. The TC plantlets have a high establishment rate in the field. Plantlets are resistant and help reduce the cost of controlling foliar diseases by at least 50%. The homogeneity of their development avails the farmer the opportunity to be in charge of routine developments in the plants such as flowering and harvesting time, resulting in a significantly improved yield output and quality (Hwang et al. 1984). At the preliminary stage of development, tissue culture plantlets are more susceptible to herbicide tolerance than sword suckers. Because labor costs are high, weed control in large farmlands by hand hoeing is impractical. Thus the indiscriminate use of herbicides will result in serious plant damage. Mature plants developed from tissue culture plantlets will produce *high mat* or exposed roots thus making the plants vulnerable to lodging and wind action after shoot development and possible topple over.

7.3.5 Use of Somaclonal Variation

Somaclonal variation as a tool for genetic improvement of plantain germplasm derived from shoot-tip has also been extensively explored, but with limited success (Vuylsteke et al. 1996). Plantain suckers were subjected to in vitro shoot-tip culture to produce true-to-type somaclones. Field performance of the somaclonal variants were evaluated for development of multiple bunching horticultural traits. Somaclones from the first, second and third ratoon crops produced the same number of multiple bunches, similar to the parent stock, were regenerated, multiplied and distributed (Vuylsteke 2000).

7.3.6 Cryopreservation of Musa Germplasm

Cryopreservation refers to the storage of biological samples at an ultra-low temperature (−196 °C) in liquid nitrogen. Cryopreservation techniques may also serve as a pathogen eradication tool. Meristem clumps of in vitro cultures regenerated from viral-infected plantain plants were exposed to the ultra-low temperature in liquid nitrogen to reduce viral infections. The procedure for subculturing is strenuous and provides an opportunity for plants to become infected by any pathogenic microbial contaminants. In addition, plants established in vitro, especially under reduced growth conditions, are prone to exhibit somaclonal variation. Overcoming these limitations and achieving long-term conservation of *Musa* germplasm resources and biodiversity is supported by cryopreservation research and storage. This cryopreservation method ensures cost-effective, safe and long-term storage of genetic resources of species which are not seed-bearing and are vegetatively propagated, such as *Musa*. This research was earlier developed and carried out at the Katholieke Universiteit Leuven, Belgium (KULeuven) and the techniques developed are now being utilized for routine cryopreservation of accessions held in different plant biodiversity collections. About one-half of the plant germplasm is at the moment preserved safely in liquid nitrogen for long-term storage. Cryopreserved collections are considered complementary to in vitro collections and serve as safe gene banks in case genotypes are lost due to impurities, somaclonal variation or laboratory mistakes arising from subculture procedures (Roux et al. 2003).

Approaches in cryopreservation are routinely applied to any type of plant tissue with high potential for regeneration. Model protocols have now been developed for over 200 diverse plant species, nurtured in different forms such as cell suspensions, calli, apices, somatic and zygotic embryos. Two types of meristematic and regenerative in vitro tissue can be obtained from plantain: (i) individual meristems obtained from shoot-tip cultures and (ii) highly proliferating meristem cultures containing *cauliflower-like* meristem clusters. Cryopreservation methods exist for both tissue types. In addition, embryogenic cell suspensions of different cultivars belonging to distinct genomic groups are also now being stored in liquid nitrogen (López et al.

2005b). The primary purpose of preserving embryogenic cell suspensions of plantain for the long term is not the conservation of *Musa* diversity. Some plantain accessions are recalcitrant toward the establishment of embryogenic cell suspensions and moreover this process is extremely time consuming (usually 15 months); cryopreservation should be considered in this case as an aid for biotechnological applications such as genetic engineering (López et al. 2005a). The merits and demerits of each protocol are explained and areas not properly addressed are further explored with a view to further optimize all the methods identified. The availability and the nature of the recipe used, the accessions undergoing cryopreservation and resource availability will have to be taken into consideration so as to determine which of these methods are most suitable for use in other laboratories (López et al. 2005b; Roux et al. 2003).

7.3.7 Production of Virus Free Plantain

Virus-infected planting materials are a major problem in plantain cultivation, exchange and storage of plantain germplasms. Most *Musa* gene banks are severely affected by viral diseases. Viral resistance can confer by the transgene in plantain; genetic manipulation in plantain, including the introduction of viral resistance, has been reviewed several times. Therefore, viral elimination therapies like meristem tip culture, chemotherapy and cryotherapy to produce virus-free *Musa* planting material are pertinent. Some of the efficiency of in vitro approaches used for viral eradication in other crops are discussed at the end of this section. In vitro culture of apical meristems or shoot tips are the best choice for explant initiation of in vitro clonal propagated selected *Musa* genotypes. The meristem-tip culture technique is generally considered a tool for virus elimination. However, the meristem culture technique is not able to remove CMV (cucumber mosaic virus) from plantain plants. In another report, few virus-free plantlets could regenerate via in vitro meristem culture technique from BSV (banana streak virus) infected plantain plants. Further, this technique was unable to recover even a single BBTV-free (banana bunchy top virus) *Musa* cultivar. Several other researchers also reported the inefficiency of the tissue culture technique to eradicate viruses in *Musa*. Therefore, other therapies in combination with the in vitro meristem-tip culture technique were screened to regenerate virus-free planting material. Furthermore, the plant tissue culture process itself may induce excision of BSV integrated sequences to cause BSV infection (Jain et al. 2007).

Furthermore, antiviral molecules adefovir, tenofovir or 9-(2-phosphonomethoxyethyl)-2,6-diaminopurine (PMEDAP) containing culture medium was used to target viral reverse transcriptase for a duration of 6 months. The treated ones were cultured to regenerate plantlets. Six-month-old plantlets in the greenhouse exhibited 69, 88 and 90% BSV eradication after PMEDAP, adefovir or tenofovir treatments, respectively. Thus, chemotherapy of the infected in vitro cultures was found quite efficient for the elimination of BSV. Chemotherapy may prove

effective for the other viruses as well in future viral disease eradication programs (Roux et al. 2003).

7.3.8 Induced Mutation in Musa Species

This section looks at a procedure which involves the use of embryogenic cell suspensions (ECS) in plantain (*Musa paradiasiaca*) using both the in vitro gamma irradiation and plant regeneration approaches in order to attain maximum genetic improvement. The procedure involves an array of activities to properly select embryonic cell suspension for irradiation and the handling of posttreatment plant regeneration in addition to mutant selection through the acclimatization process and under field conditions (Xu et al. 2012). Mutation-induced plantlets will unmask a recessive phenotype by either using mutation processes, inhibition or deletion of the homozygous dominant allele (Jain and Swennen 2004). The success of in vitro mutation breeding depends on the development of reliable and viable in vitro plant regeneration protocols, optimization and types of mutagens used as well as the efficient screening of the mutation inbred lines for the desired variations (Xu et al. 2012). Thus, somatic embryogenesis offers an efficient protocol for clonal propagation and mutation induction in plantain. Somatic embryos originate from a single cell, preventing somaclonal variations in regenerated plantlets and makes them an excellent tool for mutation breeding. Research has also revealed that somatic embryos emerging from plantain embryonic cell suspensions in most cases possess a single-cell origin. Thus, the merit of utilizing embryonic cell suspension for mutagenesis would best be obtained either in nonchimeric populations or the quick dissociation of the chimeric sectors on emergence (Roux et al. 2001).

7.3.9 Haploid Production in Plantain

The regeneration of haploid plants by the classical method of anther culture (androgenesis) is employed in various crops. It requires the evaluation of several generations, which is difficult in false fruit crops like plantain. Research reveals that the most effective means of haploid plants production using anther culture of plantain is by adopting flow cytometric procedures for polyploid determination (Jain et al. 2007).

Plantains with haploid genotypes were developed from the anther callus of plantain *Musa paradisiaca*. The highest rate of callus induction of about 90% was obtained at 2,4-D concentration of 3 mg/L. After 3 weeks of incubation, embryonic cells were developed from the callus mass. A combination BAP at 4 mg/L and indole acetic acid at 0.4 mg/L induced shoot growth of the embryonic cells and well developed roots system at a concentration of 0.6 mg/L NAA with an augmented media of 0.2% activated charcoal. The results of the investigation showed that the

protocol was efficient in the production of haploid plants from anther culture (Roux et al. 2003).

Analyses of flow cytometry was carried out by determining the intensity of leaf DNA of the in vitro regenerated plants. Jain et al. (2007) showed that identification of plantlets with lower nuclear DNA intensity value was as a result of the prevalence of aneuploid plants. This result was based on the assumption that many of the chromosomal changes were identified by the flow cytometric method were associated with variations in the number of chromosomes. These variations were capable of inducing total or partial loss of chromosomes during the doubling process (Roux et al. 2003). Flow cytometric studies thus provide the basis for other investigations to detect plants with values of nearly twice the nuclear DNA content, suggesting possible in vitro polyploidization.

7.4 Plantain Diversity in Cross River State

The biological diversity of plantain (*Musa paradisiaca*) can be viewed from three perspectives:

(a) Genetic diversity—which has to do with the heritable variations in genes and genotypes of the plantain germplasm availability in Cross River state;
(b) Plantain (*Musa paradisiaca*) species diversity—which has to do with the plantain species richness in Cross River state (elite cultivars that are mostly cultivated);
(c) Ecosystem diversity of plantain which takes into consideration the ecosystem (ecology), that best suits and favors the cultivation and growth of plantain (elite cultivars) germplasm in Cross River state.

The importance of biodiversity to mankind cannot be overemphasized. It has paved the way for the sustenance of the socioeconomic systems in ways that allow the poorest of the poor to obtain their food and nutritional needs while retaining their cultural diversity as a people (Tingey et al. 1994).

Plantain (*Musa paradisiaca*) is an important food crop with the most diverse uses in the tropics; it is seen as a very important aspect of food security and provides a reliable source of income to rural agrarian localities due to its excellent morphological attributes (Table 7.1). Plantain is undoubtedly one of the most important staple plant based foods and one of the oldest cultivated fruit crops in Sub-Saharan Africa, Central Africa, South America and Asia (Swennen et al. 1995). Plantain cultivation is undoubtedly an activity of important from a socioeconomic standpoint in the rainforest zone of Nigeria by ensuring food availability, job creation and sustainability, and it dominates the farming communities and remains a very important source of rural income and is an economic mainstay (Oritz and Vuylsteke 2002).

Plantain production remains a promising agricultural investment with enviable economic returns in Central and West Africa region and South America, which remain the major core plantain growing regions globally. It is one of the few most

Table 7.1 Morphological attributes of elite plantain accessions cultivated in Cross River State

Elite plantain accessions	Length of cycle (months)	Shape	Bunch weight (kg)	No. of hands/ bunch	No. of fingers/ hand	No. of fingers /bunch	Finger skin color	Pulp color	Harvest interval (days)	Fingers cross section	Pseudostem color	Bunch pheno-type	Hardness (kg/cm^3)	Weight ratio
Ogoni red French	15	Medium /curve	13.23	6	4	24	Red	Brown	69	Triangular	Purple	French type	1.9–1.7	1.3–1.5
Kigwa brown false horn	14	Medium/ curve	12.01	4	4	16	Brown	Milky white	62	Quadrilateral	Gray	False horn	1.8–1.6	1.3–1.5
Enugu black false horn	15	Big / curve	23.54	8	8	64	Dark green	Creamy	71	Pentagonal	Green	False horn	1.4–1.1	1.2–1.3
Ebi egome false horn	14	Big /flat	22.13	9	9	81	Pale green	Creamy	74	Quadrilateral	Green	False horn	1.4–1.1	1.2–1.3
Owomoh true horn	13	Medium /flat	15.79	6	5	30	Pale green	Milky white	65	Triangular	Gray	True horn	1.7–1.5	1.3–1.3
Kenkwa false horn	14	Medium /curve	16.23	7	6	42	Pale green	Creamy	66	Pentagonal	Brown	False horn	1.3–1.0	1.02–1.15
Kainjen false horn	15	Big /flat	19.88	8	6	48	Olive green	Whitish	62	Quadrilateral	Brown	False horn	1.7–1.5	1.3–1.4
Uhom false horn	13	Medium /curve	14.23	7	5	35	Pale green	Creamy	67	Quadrilateral	Green	False horn	1.7–1.5	1.3–1.4
Ekumkwam French	14	Medium /flat	16.22	9	8	72	Dark green	Creamy	63	Triangular	Green	French type	1.1–0.9	1.14–1.3
Ikpobata French (cooking bananas)	13	Small / curve	7.21	4	4	16	Dark green	Milky white	69	Triangular	Brown	French type	1.9–1.7	1.5–1.7
Mgbeghe false horn	14	Big /flat	24.97	6	7	42	Pale green	Creamy	73	Quadrilateral	Green	False horn	1.9–1.7	1.4–1.6

(continued)

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Elite plantain accessions	Length of cycle (months)	Shape	Bunch weight (kg)	No. of hands/ bunch	No. of fingers/ hand	No. of fingers /bunch	Finger skin color	Pulp color	Harvest interval (days)	Fingers cross section	Pseudostem color	Bunch pheno-type	Hardness (kg/cm^3)	Weight ratio
Ingwam French	17	Medium /curve	15.64	6	5	30	Pale green	Creamy	65	Quadrilateral	Brown	French type	1.7–1.5	1.2–1.4
Bakpri French (dwarf mutant)	16	Small / curve	5.11	3	3	9	Dark green	Milky white	61	Triangular	Green	French type	1.8–1.6	1.4–1.6
Ejorgom True horn	15	Medium /curve	13.80	6	5	30	Pale green	Creamy	68	Pentagonal	Brown	True horn	1.7–1.5	1.3–1.5

Source: Ubi et al. (2016)

important sources of energy diet in most areas where it is cultivated and used as a main staple food crop by more than a billion people globally (Ray et al. 2006).

Plantain is a tropical crop which originated in Southeast Asia with a wide variety found in the area of Myanmar. It is a monocot plant with parallel venation. It is an herbaceous crop which matures within a year or two, but is naturally a perennial crop, because the suckers continue to produce. It does not have a woody stem. The true stem is found underground and is known as the rhizome. It has many eyes or buds, which grow into suckers.

Nigeria is one of the major plantain producing countries of the world, which is partly fueled by the higher rate of utilization of plantain products in the country due to the ever-increasing population and their need for sustenance (Racharak and Eiadthong 2007). Nigeria is the largest producer of plantain in West Africa, producing more than 64% of the total plantain in the sub region. Cross River state in Nigeria is the highest producer of plantain with almost all the local government areas of the state serving as hubs for plantain trade and distribution.

Plantain genetic resource conservation merits far greater attention than it is presently receiving due its wide economic advantage over most other indigenous/local food tree crops, arising from its non-seasonality, adaptability to the wide range of ecological habitats prevalent in Cross River state, availability of high yielding elite cultivars, ease of cultivation, availability of markets, high demand, availability of good postharvest processing techniques, better shelf life of products and good farm gate prices.

These factors have endeared the elite plantain (*Musa paradisiaca*) cultivars to Cross Riverians and thus enhanced the realization of the productive potentials of plantain by farmers. This has also created a heightened need for this discussion which intends to highlight the ecological biodiversity of elite plantain (*M. paradisiaca*) cultivars available in Cross River state in terms of genetic diversity, plantain species diversity and spread as well as ecological system diversity of the elite plantain germplasms in the state.

7.5 Molecular Characterization of Elite Plantain Germplasm in Cross River State

Plant genetic resources are among the most essential of the world's natural resources. Variability in genomic DNA sequences of plant cultivars have played a major role in varietal characterization and improvement of many crops species, including plantain, and have contributed to the capacity to assess biodiversity, evaluate phylogenetic relationship and estimate potential yield capacity (Alvarez 1997; Pillay et al. 2000).

Genetic diversity is the genetic variability existing among individuals of a species of the same genus (Nei 1978). Genetic diversity derives from the various

genetic differences or polymorphisms between and among individual species and may unfold in variations in DNA nucleotide sequences, in biological and physiochemical attributes (as seen in protein structure or isoenzyme properties), in chemical properties (temperature, nutrient deficiency, and other abiotic stress resistance or growth rate) and in phenological attributes such as plant height or color of unripe fruit (Ruangsuttapha et al. 2007).

Genetic diversity in plantain can be evaluated in terms of the different forms (alleles) as may be found in the different populations, their distribution and the overall distinctiveness between different populations. The variation that underscores genetic diversity in the elite plantain cultivars in Cross River state arises from mutations and recombination (Agoreyo et al. 2008). Selection, genetic drift and gene flow are among the indices of the alleles present in different populations which induce variation. It is generally believed that genetic variability in a plant population is structured in space and time (Lakshmanam et al. 2007).

Presently, conservation efforts for plantain germplasm include in situ and ex situ measures, which have proceeded with little or no information on genetic diversity. Gene banks have also been established for storage of plantain germplasm in the form DNA sequences in most of the biological databases like the National Centre for Biotechnology Information and the European Molecular Biology (IITA 2018).

Thus genetic diversity remains one of the sole bases for speciation, adaptation and survival, thereby preempting the possibility to advance the foundations upon which evolutionary relatedness and the survival of human depend. Hence, the knowledge of the genetic diversity of plantain biodiversity in Cross River state will help in the preservation of the rich and available plantain germplasms in gene banks and other in situ conservation media to prevent the loss of plantain biodiversity occasioned by biotic and abiotic stresses, competition, predation, parasitism, pests and diseases, isolation, habitat alteration, urbanization, climate change, natural disasters and human advancement. Thus it has become imperative, given this threat, to fully understand and utilize the knowledge of genetic diversity in the conservation of plantain genetic resources for food security, income generation and agricultural developmental sustainability.

Studies by Ubi et al. (2016) revealed the existence of sufficient amounts of genetic diversity among the elite plantain cultivars in Cross River state. Fourteen elite plantain cultivars sampled from the state and subjected to molecular analysis using microsatellite markers or simple sequence repeat (SSR) are given in Table 7.2; the markers in Table 7.3:

(a) 55 mean total number of amplified bands
(b) 65.47 mean % polymorphism
(c) 0.67 mean polymorphic information content (PIC)
(d) 3.54 mean marker option index
(e) 0.832 mean gene diversity
(f) 0.11–0.91 genetic distance range

Table 7.2 Selected microsatellite primers used for the 14 elite plantain (*Musa paradiasiaca*) genotyping

S/N	Primer	Motif	References	Annealing temp. (°C)
1	Ma-1-32	$(GA)_{17}AA(GA)_8AA(GA)_2$	Crouch et al. (1998)	58
2	Ma-3-90	$(CT)_{11}$	Crouch et al. (1998)	53
3	mMaCIR 307	$(CA)_6$	Hippolyte et al. (2010)	54
4	mMaCIR 264	$(CT)_{17}$	Hippolyte et al. (2010)	53
5	mMaCIR 260	$(TA)_8$	Hippolyte et al. (2010)	55
6	mMaCIR 39	$(CA)_5GATA(GA)_5$	Lagoda et al. (1998)	52
7	mMaCIR 196	$(TA)_4\ (TC)_{17}\ (TC)_3$	Hippolyte et al. (2010)	55
8	mMaCIR 214	$(AC)_7$	Hippolyte et al. (2010)	53
9	mMaCIR 01	$(GA)_{20}$	Lagoda et al. (1998)	55
10	mMaCIR 03	$(GA)_{13}$	Lagoda et al. (1998)	53

Source: Ubi et al. (2017a, b)

The reports also showed that the 14 elite plantain cultivars in Cross River state are genetically grouped into 4 distinct clusters as shown in Fig. 7.2.

Microsatellite markers like simple sequence repeats (SSR) are more informative in the identification and discrimination of closely related genotypes and are considered a great asset in fingerprinting, mapping, and genotyping of plants, and can be used for distinguishing within and between the groups of plantains evaluated in this study. Hence, simple sequence repeat markers have been fully utilized for the purposes of genetic analysis, including genetic diversity (Fig. 7.3 and Table 7.3) of plants. It also used for the determination of the relationships between plant accessions, elucidation of evolutionary relationships (Fig. 7.4), as an instrument in the taxonomic classification of many plants including tropical pasture grasses, as well as in the molecular genotyping of important crops such as rice, orphan legume species, banana and plantain. Also, their use in the evaluation of dichotomous bunching plantain cultivars is highly valuable and highly recommended as this will help to pinpoint the degree of divergence within them on the one hand and between them and the single bunching cultivars of False Horn plantains on the other. In addition, utilizing molecular markers which can help to identify many genetic polymorphisms would equally make it feasible to address the relationship, if any, between morphological and genotypic variations among multiple (dichotomous) bunching plantain varieties that consequently will provide substantial insights in directing introgression and molecular breeding strategies in these varieties of plantain.

Table 7.3 Genotyping of the 14 Elite Plantain accessions using the selected microsatellite markers

S/N	Marker	Major allele frequency	Number of allele	Heterozygosity	Mean allele frequency	Coefficient of gene differentiation	Gene diversity	Polymorphic information content
1	mMaCIR 01	0.105	18.00	0.543	0.405	0.364	0.886	0.942
2	mMaCIR 03	0.357	10.00	0.400	0.654	0.433	0.765	0.694
3	Ma-3-90	0.167	15.00	0.474	0.543	0.436	0.852	0.913
4	Ma-1-32	0.215	12.00	0.493	0.665	0.555	0.831	0.876
5	mMaCIR 307	0.476	8.00	0.321	0.772	0.421	0.635	0.533
6	mMaCIR 264	0.239	18.00	0.522	0.475	0.678	0.836	0.874
7	mMaCIR 260	0.329	14.00	0.357	0.609	0.628	0.762	0.685
8	mMaCIR 196	0.250	13.00	0.453	0.584	0.546	0.829	0.855
9	mMaCIR 39	0.200	18.00	0.531	0.665	0.455	0.860	0.893
10	mMaCIR 214	0.383	9.00	0.365	0.576	0.626	0.772	0.670

Source: Ubi et al. (2017a, b)

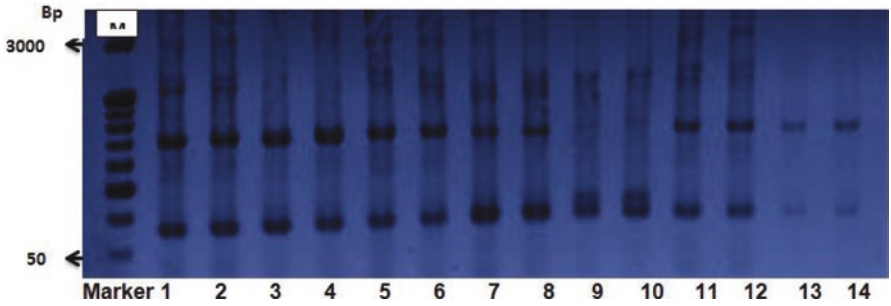

Fig. 7.2 Electropherogram showing amplified fragment bands of 14 elite cultivars of plantain amplicons in agarose gel using microsatellite SSR markers. Arrows in gel photo indicates the range (50–3000 bps) of size of the molecular marker (DNA ladder). Numbers 1–14 represents the different 14 plantain cultivars evaluated as presented in Table 7.4. (Source: Ubi et al. 2017a, b)

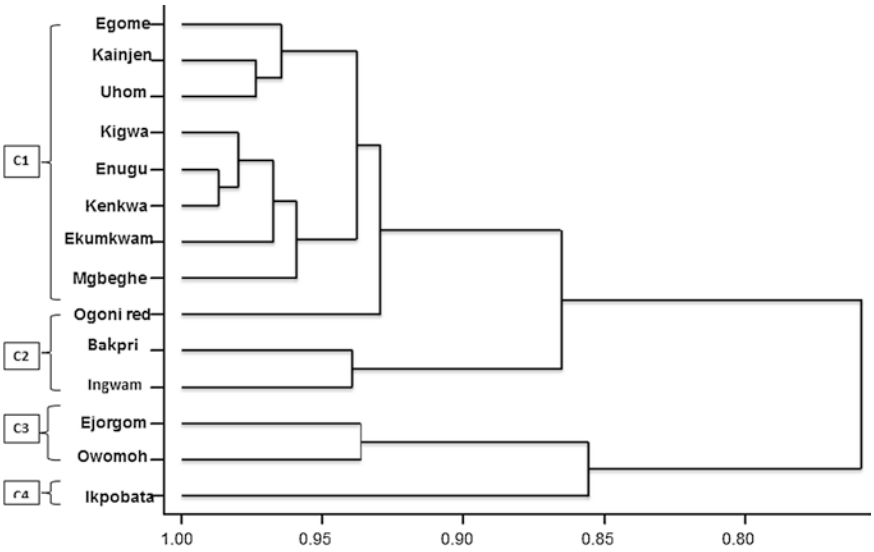

Fig. 7.3 Dendrogram showing genetic diversity showing among 14 elite plantain (*Musa paradisiaca*) cultivars in Cross River state. (Source: Ubi et al. 2017a, b)

7.6 Molecular Phylogeny of Species Diversity of Plantain in Cross River State

The phylogenetic tree above represents the evolutionary tree and pathway followed by the elite cultivars of plantain (*Musa paradisiaca*) in the study area. The phylogenetic tree is composed of branches, also known as edges, which connect and terminate at nodes.

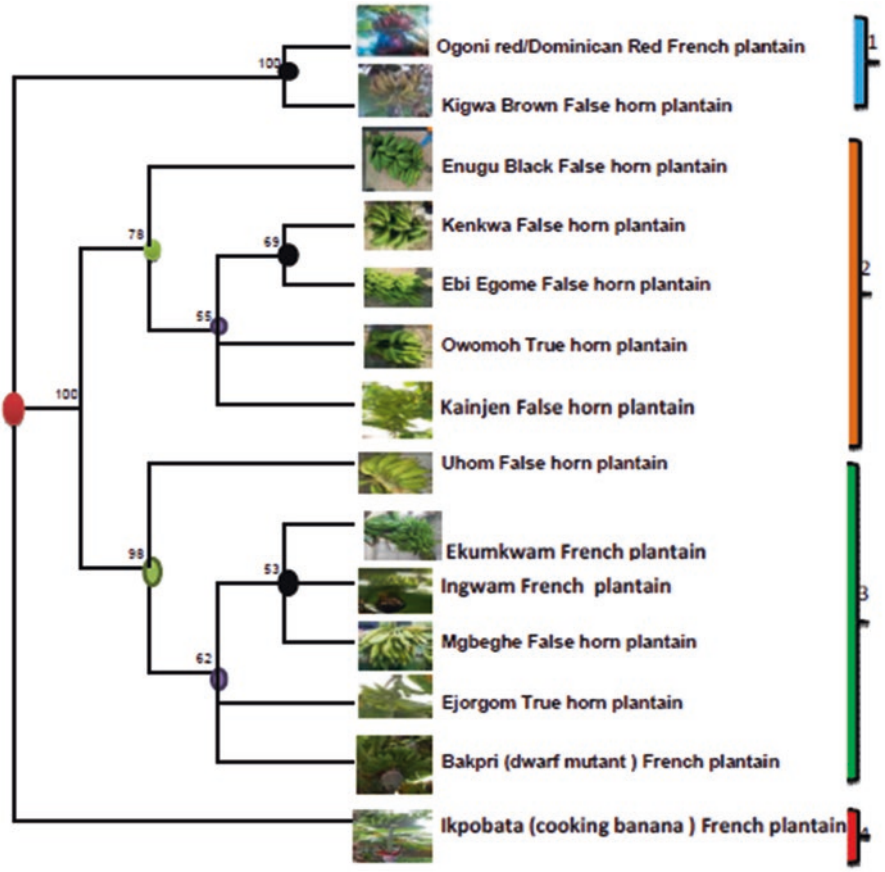

Fig. 7.4 Phylogenetic tree inferring evolutionary relationship on elite plantain cultivars grown in Cross River state. (Source: Ubi et al. 2017a, b)

- This color indicates the branches and nodes which can be internal or external (terminal) as observed from the phylogenetic tree (Fig. 7.4). The branches length varies and represents the evolutionary pathway and time of evolution. Longer branches indicates longer period of evolutionary development and mutation while shorter branches indicates shorter period of evolutionary development between and among the plantain species.
- This color indicates the terminal node at the tips of the tree and represents the operational taxonomic units (OTUs). OTUs corresponds to the molecular sequences or taxa (species) from which the tree was inferred.
- This color indicates the internal nodes and represents the last common ancestor (LCA) to all nodes that arise from that point.
- The red color indicates the tree root which emanated from a single gene from many taxa (*Musa* spp.) and through evolutionary sequences and mutation changes developed into multigene families (gene tree).

The edge length represents mutation events which are supposed to have occurred on the evolutionary path. Variations in edge dimensions in the phylogenetic tree shows the rate at which mutations accumulate in the sequences varies among the cultivars or the taxa.

7.6.1 The Operational Taxonomic Units (OTUs) and the Branches

Plantain cultivars used here represent the leaves and are referred to as taxa or OTUs. The phylogenetic tree was reconstructed for this set of taxa, using their respective genes or protein sequences (Fig. 7.4). The OTUs species in the first cluster have a 100 G consensus with only two relatives, the species in the second cluster also have two relatives extending to develop into branches and have a 69 G consensus while the third cluster group has three relatives extending to the branches with 53 G consensus. OTUs can be used to build an unrooted phylogenetic tree that clearly depicts a path of evolutionary changes (Ubi et al. 2017a, b).

7.6.2 The Clades

These are a group of OTUs that include several sequences and their common ancestor nodes. In the above phylogeny tree, 2 clades are represented in the tree comprising 5 cultivars of plantain as seen in cluster 2 and 6 cultivars of plantain as seen in cluster 3 above. In the first clade which is represented as cluster 2, there are 2 branches (cultivars) with a first internal node or last common ancestor and a second descendant of its neighboring close relative linked to the clade (Fig. 7.4).

The second clade which is represented as cluster 3 has 3 branches (cultivars) with first internal node or last common ancestor and a descendant of its neighboring relatives linked to the clade (Ubi et al. 2017a, b).

7.6.3 The OTU Group

At times it is possible to gather information on the evolutionary relationships of a particular ingroup from a more distant taxon, unrelated to the other taxa. This type of taxon is called an outgroup. Hence, the addition of a root node to the edge and then to the outgroup, then allows interpretation of bifurcations with regard to the time of divergence. An OTU group therefore is for the purpose of finding the root of the tree. In the phylogenetic tree above, the cooking banana cultivar with an ABB genome is distantly related to all other cultivars in the study with AAB genomes. This cultivar therefore was basically used to obtain the common root or ancestry for the 14 elite cultivars of plantain used for the study (Fig. 7.4).

A tree is said to be rooted if there is a single node or outgroup that is an external point of reference point from which all OTUs in the tree arise. The root is the oldest point in the tree and the common ancestor of all taxa in the analysis. In the absence of a known outgroup, the root can be placed in the middle of the tree or a rootless tree may be generated.

In molecular phylogeny, trees are drawn so that branch length corresponds to the amount of evolution that is the percent difference in molecular sequences between nodes. Once a gene has been duplicated, all other subsequent species in the phylogeny will inherit both copies of the gene to create orthologs as shown in clusters 1, 2 and 3 above from the phylogenetic tree.

The monophyletic groups consist of an internal last common ancestor (LCA) node and all OTUs arising from it. All members within the elite plantain cultivar groups here presented were derived from a common ancestor and have inherited a set of unique common traits. On the other hand, polyphyletic groups are an assemblage of distantly-related OTUs that possess similar characteristic or phenotype, but are directly not descendants from a common ancestral parent.

Almost all phylogenetic tree reconstruction methods reconstruct an unrooted binary tree which cannot be interpreted with respect to a time scale. In an unrooted tree one may not know if an internal node is the LCA or the descendant of its neighboring adjacent node.

Interestingly, evolutionary divergence species may result in many variations of protein, all with similar structures and functions, but with very different sequences. Bioinformatics and phylogenetic studies have been widely used to trace the origin of protein sequences to an ancestral protein family or gene line. Molecular sequence may evolve over time as a result of multiple sequence or gene mutations that result in small, but evolutionarily-important changes in the nucleotide sequences. At the protein level, these may not initially affect protein structure or function, but over time, may eventually shape a new purpose for a protein within different species.

This does not apply in all findings, as all elite cultivars have descended from a common ancestor. An evolutionary event is shaped by homology, which describes any similarity arising from the possession of common ancestry. Furthermore, phylogenetic trees are defined in terms of their homologous relationships. Paralogs are homologous sequences that are separated by a gene duplication event while orthologs are homologous sequences are those that are separated by a speciation event when one species separates or diverges into two. Paralogs are created by the duplication of gene events.

7.7 Plantain Species Diversity and Cultivation Type

Studies by Ude et al. (2003) revealed that in Africa, 116 cultivars of plantain (*Musa paradisiaca*) exist and these vary greatly from one country to another as well as from one geographical locality to the other.

Until recently, identification of these wide varieties of plantain has traditionally been based on morphological criteria (Reddy et al. 2002). However, this does not reveal the close genetic relationship and molecular characteristics that exist among the identified cultivars occasioned by frequent somatic mutations and morphological changes due to environment, thus posing a major obstacle to the proper identification of existing cultivars (Delaporta et al. 1983). This triggers the call for genetic diversity studies using molecular markers to complement the use of morphological characteristics in the identification of existing cultivars in Cross River state.

A wide range of plantain cultivars are grown in Cross River state. Local cultivars with names such as Ukom, Uhom, Egome, Ejorgom, Owomoh, Ingwam, Kenkwa, Kainjen, Mgheghe, are some of the most prominent (elite) cultivars of plantain cultivated in the state (Ubi et al. 2016). Botanically, plantain cultivars in Cross River state exhibit a triploid cytogenetic structure with chromosome number 2n = 22 with an AAB Genome. These cultivars belong to the following types.

7.7.1 True Horn Plantain

True Horn (Fig. 7.5a) bears a bunch with fewer hands and fruits, but individual fingers are very large. It possesses an incomplete inflorescence. There is a complete absence of hermaphrodite flowers and a male floral bud. The horned plantain (Table 7.4) possess incomplete bunches at maturity, that is, the fingers and hands do not fill up or complete all the available buds in the inflorescence (Simmonds 1959).

7.7.2 False Horn Plantain

According to Stover and Simmonds (1987), False Horn (Fig. 7.5b) bears a bunch with increased number of hands and fruits, but individual fruits or fingers are small compared to the True Horn type. The False Horn plantain (Table 7.4) also possesses an incomplete inflorescence. However, this type of plantain contains only a few hermaphrodite flowers and the remains of the male floral buds. False Horn and True Horn are found extensively in Ivory Coast where they represent 91–96% of the country's plantain production.

7.7.3 French Plantain

French Plantain (Fig. 7.5c) bears a bunch containing many hands with an increased number of fruits per hand, but the fruits are smaller than the horn types. It bears a complete inflorescence with the presence of both hermaphrodite flowers and a

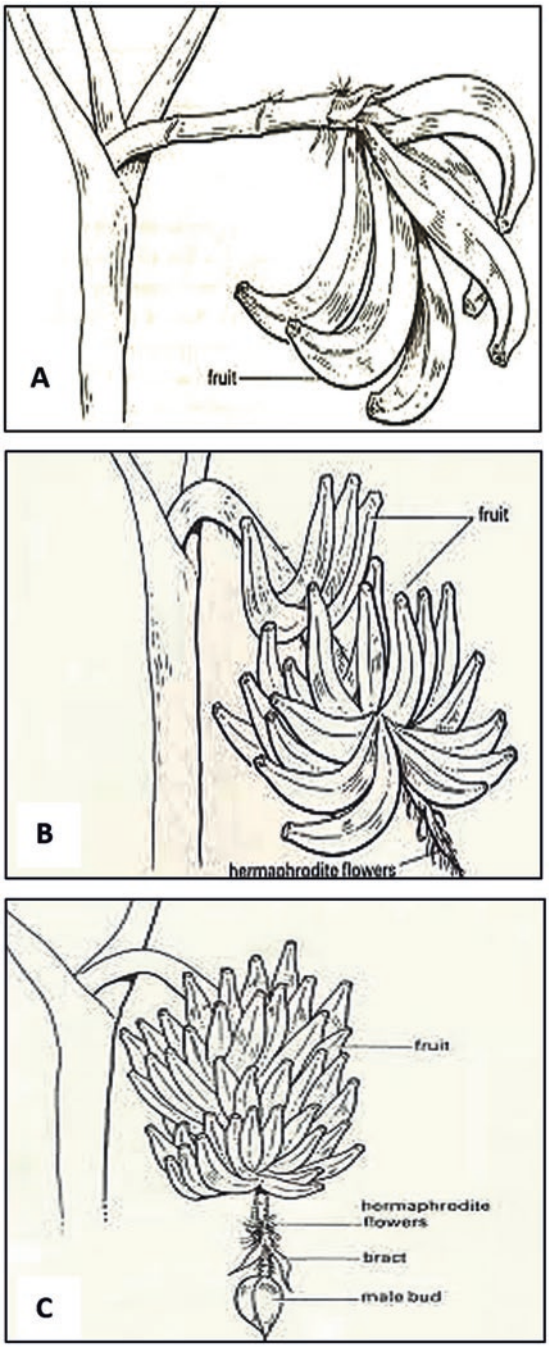

Fig. 7.5 Species diversity showing the True Horn (**a**) False Horn (**b**) and French Plantain (**c**) types in Cross River state. (Source: Ogazi 1996)

Table 7.4 Elite plantain showing species diversity in Cross River State

S/N	Elite plantain cultivar (local name)	Plantain species types	Genome
1	Ogoni red plantain	French	AAB
2	Kigwa brown plantain	False horn	AAB
3	Enugu black plantain	False horn	AAB
4	Ebi egome plantain	False horn	AAB
5	Owomoh plantain	True horn	AAB
6	Kenkwa plantain	False horn	AAB
7	Kainjen plantain	False horn	AAB
8	Uhom/Ukom plantain	False horn	AAB
9	Ekumkwam plantain	French	AAB
10	Ikpobata (cooking banana)	French	ABB
11	Mgbeghe plantain	False horn	AAB
12	Ingwam plantain	French	AAB
13	Bakpri plantain	True horn	AAB
14	Ejorgom plantain	True horn	AAB

Source: Ubi et al. (2016)

persistent male floral bud. The French Plantain (Fig. 7.6 and Table 7.4) is extensively found in the coastal regions of West Africa, Cameroun, Rwanda and Burundi (Tezanas 1987).

According to Swennen and Vuylsteke (1987), bunch phenotype determines yield and fruit quality and is considered the most striking morphological trait for differentiation of clones. In the French Plantain, the male flowers persist until the bunch matures. The bunch is complete at maturity.

Studies by Ubi et al. (2017a, b) reveal that False Horn plantains are the dominant cultivar in Cross River state constituting up to 54%, followed by the French Plantain with 26.0%, while the True Horn plantain is the least cultivated with only 20% of production.

7.8 Plantain Ecosystem Diversity in Cross River State

Ecological and geographical differences in the distribution of plantain biodiversity (genetic, species, ecological diversity) are extremely common. In fact, differences in geographical distribution are nearly almost impossible to separate from ecologically-induced variations. Different geographical localities will always differ with respect to some potentially significant ecological characteristics such as latitude, longitude, temperature and moisture availability. Several studies have clearly demonstrated that there is a strong association between plantain population characteristics and the environments in which they grow (Ubi et al. 2017a).

Ecological factors tend to play a very significant role in determining the extent and distribution of biodiversity in plantain and their wild relatives (Venkatachalam

Fig. 7.6 Fourteen elite plantain cultivars. Names are listed in Table 7.4. (Source: Ubi et al. 2016)

et al. 2008). Ecological differences in plantain affects many traits in plantain such as the relative rate of development, resistance to biotic and abiotic stresses, edaphic responses to soil fertility and adaptation to cultivation, irrigation, quality differences, as well as methods of harvesting and utilization (Venkatachalam et al. 2007). Thus, the resultant traits or attributes of the plantain crop are a combination of climatic, soil or edaphic factors and breeding system. This explains why different communities and local government areas maintain a greater or lesser number of different types of plantain (Fig. 7.5 and Table 7.5) (Ubi et al. 2017b).

Table 7.5 Cultivars of plantain showing ecosystem diversity in Cross River state with coordinates readings showing areas of high population/ecological diversity

S/N	Plantain cultivar	Latitude (N)	Longitude (E)	Elevation (m)	Area of least diversity (LGA)
1	Ogoni red	06° 54. 58′	09° 17. 79′	178	Bendi II (Obanliku)
2	Kigwa brown	06° 48. 62′	09° 15. 72′	183	Baterico (Boki)
3	Enugu black	06° 02. 84′	08° 41. 11′	49	Nde (Ikom)
4	Ebi egome	05° 56. 54′	08° 50. 46′	131.4	Etomi (Etung)
5	Owomoh	05° 55. 88′	08° 26. 01′	175	Ochon (Obubra)
6	Kenkwa	06° 04. 45′	08° 54. 77′	129	Bashua (Boki)
7	Kainjen	05° 58. 20′	08° 63. 52′	181	Mkpani (Yakurr)
8	Uhom	05° 40. 19′	08° 03. 52′	56	Akpet 1 (Biase)
9	Ekumkwam	06° 38. 46′	08° 52. 29′	110	Mbube (Ogoja)
10	Ikpobata	06° 28. 43′	09° 08. 84′	97	Agoi Ekpo (Yakurr)
11	Mgbeghe	05° 38. 71′	08° 48. 02′	119	Eku (Akamkpa)
12	Ingwam	06° 39. 99′	08° 51. 60′	92	Abuochichie (Bekwarra)
13	Bakpri	04° 97. 78′	08° 36. 01′	54	Awi (Akamkpa)
14	Ejorgom	06° 30. 72′	09° 10. 69′	118	Ukelle (Yala)

Source: Ubi et al. (2016)

7.9 Inflorescence Developmental Polymorphism in Plantain

This section discusses the occurrence and persistence of inflorescence developmental polymorphism (multiple bunching) in plantain. It also highlights the molecular and genetic characteristics of the different forms of inflorescence developmental polymorphism in plantain using DNA-based molecular markers such as the simple sequence repeat (SSR or microsatellites) and single nucleotide polymorphism (SNP).

Inflorescence developmental polymorphism or multiple bunching in plantain is a form of variation which can contribute to crop improvement, if valuable and genetically stable clones are selected and regenerated (Vuylsteke 2000). Plantains which bear several bunches have occasionally been observed and reported. Stover and Simmonds (1987) observed an unusual plant of Grande Naine with a double inflorescence of the pseudostem, with an inflorescence borne on each pseudostem.

The first detailed account of inflorescence developmental polymorphism in plantain in Africa was provided by Pospisil (1966). It is, therefore, certain that this character is invariably propagated through the suckers. The occurrence of twin fingers is a common feature of the first bunch (Karikari et al. 1971), which is also an indication that subsequent bunches will produce the inflorescence variants.

7.9.1 Advantages of Inflorescence Developmental Polymorphic Plantain

Multiple bunching plantain has several and innumerable advantages over the single bunching plantain in that the former produces many fingers per hand, many hands per bunch and many bunches per plant giving a higher yield per hectare compared to the single bunches. This multiple bunching plantain ensures increase income to plantain farmers and food security for local citizenry (Tenkouano 2000).

7.9.2 Phenotypes of Multiple Bunching in Plantain

Robinson (1996) viewed the inflorescence as a single bract spike, with a stout peduncle which bears most of the biseriate nodal clusters of flowers. Each nodal cluster is anchored by an inflorescence bract that guards the tender developing flowers. Inflorescence developmental polymorphism in plantain is a dichotomization event which can contribute to crop improvement if valuable and genetically stable clones are selected and regenerated (Vuylsteke 2000). Mutants of inflorescence polymorphic plantain types are rare. In fact, in most areas, they are cut away as soon as they are found for superstitious reasons, and this may be the cause of their scarcity. There are therefore, various identified forms of inflorescence polymorphism in plantain discussed as follows.

7.9.2.1 Double Bunching in One Peduncle (DB1P)

According to Baiyeri (1994), these are two bunches of plantain that are borne on one peduncle on a single plant (Fig. 7.7a). The two bunches may vary significantly in their hand and finger numbers per bunch as well as in their finger size. However, the two bunches are of the same bunch phenotype, False Horn, and may have the same qualitative attributes such as pulp color, finger skin color and finger orientation.

7.9.2.2 Double Bunching Borne in Two Peduncles (DB2P)

These are two bunches of plantain that are borne on two separate peduncles (one on each peduncle) on a single plant (Fig. 7.7b) The two bunches may vary significantly in their hand and finger numbers per bunch as well as in their finger sizes. However, the two bunches are of the same bunch phenotype, that is, False Horn, and may have the same qualitative attributes such as pulp color, finger skin color and finger orientation (Baiyeri 1994).

Fig. 7.7 Inflorescence polymorphic plantain types. (**a**) Double bunching plantain on one peduncle (DB1P), (**b**) double bunching plantain on two peduncles (DB2P), (**c**) triple bunching plantain on one peduncle (TB1P), (**d**) triple bunching plantain on two peduncles (TB2P), (**e**) triple bunching plantain on three peduncles (TB3P). (Source: Brisibe and Ekanem 2019)

7.9.2.3 Triple Bunching in One Peduncle (TB1P)

These are three bunches of plantain that are borne on one peduncle on a single plant (Fig. 7.7c). The three bunches may vary significantly in their hand and finger numbers per bunch as well as in their finger sizes. However, the three bunches are of the same bunch phenotype, False Horn, and may have the same qualitative attributes such as pulp color, finger skin color and finger orientation (Baiyeri 1994).

7.9.2.4 Triple Bunching in Two Peduncles (TB2P)

These are three bunches of plantain that are borne on two peduncles (two on one peduncle and one on the other) on a single plant (Fig. 7.7d). The three bunches may vary significantly in their hand and finger numbers per bunch as well as in their finger sizes. However, the three bunches are of the same bunch phenotype, False Horn, and may have the same qualitative attributes such as pulp color, finger skin color and finger orientation (Baiyeri 1994).

7.9.2.5 Triple Bunching in Three Peduncles (TB3P)

These are three bunches of plantain that are borne on three separate peduncles (one on each) on a single plant (Fig. 7.7e). The three bunches may vary significantly in their hand and finger numbers per bunch as well as in their finger sizes. However, the three bunches are of the same bunch phenotype, False Horn, and may have same qualitative attributes such as pulp color, finger skin color and finger orientation (Boumah-Mensah 1970).

7.10 Breeding Constraints in Inflorescence Developmental Polymorphic Plantain

At present, breeding efforts in *Musa* species (AAB and ABB genomes) is faced with serious challenges, especially due to the low level of fertility and seed production in several genotypes, structural incompatibility, absence of well-defined germplasm as potential stocks for breeding and the general lack of knowledge of the genetic factors responsible for important agro-morphological attributes. Expectedly, this has also affected investigations on inflorescence dichotomous *Musa* varieties. An accurately monitored and controlled scientific experiment to identify the remote causes and nature of inflorescence developmental polymorphism in plantain can go a very long way in providing and identifying the pathway underlying the cytological and molecular mechanisms responsible for controlling its expression, which will obviously be crucial for understanding this phenomenon.

Studies by Ubi and Brisibe (2017) have shown that inflorescence developmental polymorphism is multidimensional; to understand it, there are three clear goals: (a) unravel the cytological profile of these plants in terms of number, structure and behavior of mitotic chromosomes, (b) precisely determine the DNA ploidy status of a set of multiple bunching *Musa* cultivars over several crop production cycles based on chromosome counting and (c) characterize single nucleotide polymorphisms from the genomic sequences of the different inflorescence dichotomous accessions studied. These are important because such details will not only increase the knowledge of the diversity but will equally assist agronomist and plant breeders in understanding the pattern and extent of existing genetic resources available within the *Musa* genus, which can be used for exploitation in future varietal improvement programs for the crop.

7.11 Inflorescence Developmental Polymorphism (Multiple Bunching) in False Horn Plantain

7.11.1 Use of Concordance Coefficient Analysis

The concordance coefficient estimate for natural occurrence and persistence of inflorescence developmental polymorphism in plantain revealed that a unit concordance coefficient equals 1, which is an indication of normal occurrence and persistence, is observed in the single-bunching plantain only. A concordance coefficient of 0.11, far less than 1, was obtained for the plants with triple bunch borne on one peduncle, equally indicative of the random nature of occurrence and nonpersistence of the phenomenon in the accession (Ubi and Brisibe 2018).

Concordance coefficient values of zero were obtained for plants with three bunches borne on two and three peduncles, respectively, which is also an indication of the random nature of occurrence and nonpersistence of the phenomenon in the accessions. Thus, the double-bunching accessions occur more frequently than the triple-bunching accessions even though the occurrence is not persistent (Ubi and Brisibe 2018).

7.11.2 Use of Poisson Distribution Analysis

To further confirm that inflorescence developmental polymorphism phenomenon in plantain is a random event, the Poisson distribution was also used. It was gathered from the statistical inferences that Tabulated X^2 of 11.070 is greater than the Calculated X^2 of 3.013, showing that there is no significant differences between the observed and expected polymorphism. Consequently, the occurrence of

inflorescence developmental polymorphism in plantain is a random event or random occurrence which is not persistent (Ubi and Brisibe 2018).

7.12 Factors Influencing Inflorescence Developmental Polymorphism in Plantain

7.12.1 Environmental Factors

7.12.1.1 Acid Rain and Mist

The effect and influence of acid rain on the persistence of multiple bunching at 7 months of age of a first ratoon/follower crop was investigated in Calabar for three cropping cycles from 2014 to 2016. Results showed that plots treated with dilute sulfuric acid (acid rain) produced 100% persistence of all the morphotypes evaluated for the first ratoon/follower crops at 7 months of growth (Ubi and Brisibe 2017).

7.12.1.2 Crude Oil Pollution in Soils

The effect and influence of crude oil soil pollution on the persistence of multiple bunching in 7-month-old first ratoon/follower crop was investigated in Calabar for three cropping cycles from 2014 to 2016. Plots treated with crude oil as a soil pollutant produced 67% of all the morphotypes evaluated for the first ratoon/follower crops at 7 months of growth (Ubi and Brisibe 2017).

7.12.1.3 Chemical Mutagens from Industrial Effluents

The effect and influence of chemical mutagen (sodium azide, NaZ) on the persistence of multiple bunching in 7 months old first ratoon/follower crop was investigated in Calabar for three cropping cycles from 2014 to 2016. Plots treated with the chemical mutagen (sodium azide, NaZ) produced 100% of all the morphotypes evaluated for the first ratoon/follower crops at 7 months of growth (Ubi and Brisibe 2017).

7.12.1.4 High Organic Residue in Soil

The effect and influence of high volume of organic residue on the persistence of multiple bunching in 7-month-old first ratoon/follower crop was investigated in Calabar for three cropping cycles from 2014 to 2016. The plot with a high volume of organic residue treatment produced 33% of all the morphotypes evaluated for the first ratoon/follower crops at 7 months of growth. (Ubi and Brisibe 2017). These

results substantiate our earlier position that this phenomenon of multiple bunching is triggered by some environmental factors and not necessarily nutritional factors.

7.12.2 Genetic Factors

7.12.2.1 DNA Methylation

DNA methylation is a process by which methyl groups are introduced into a DNA molecule leading or giving rise to methylation. Methylation of the DNA can alter the expression or functionality of the associated genes of a DNA segment without altering the sequence orientation.

DNA methylation can also be seen as the introduction of a methyl (CH3) group to the DNA molecule, basically to the fifth carbon atom of a cytosine ring, thereby modifying the function of the *GTPase* protein binding genes thus affecting gene expression. The findings in the sequence analysis and mutation assay in the polymorphic accessions revealed that the changes in amino acids composition of the *GTPase* protein binding genes in the polymorphic accessions mostly accounted for the phenotypic plasticity observed. DNA methylation cannot be ruled out as being partly or totally responsible for the epigenetic signaling showing the phenotypic plasticity (Ubi et al. 2017a, b).

7.12.2.2 Epigenetic Control

Epigenetics is the study of the heritable phenotypic changes that do not involve alterations in the DNA sequence. A single genotype controls different phenotypes. The genotypes of the chromosomes for the polymorphic accessions are similar, especially in their chromosome numbers, but revealing different phenotypic expressions (Ubi and Brisibe 2018).

7.12.2.3 Pleitropy

This is the phenomenon that involves the influence of more than a single phenotype by a single gene. The *GTPase* protein binding gene that is a part of the *leaf tissue* complex gene that controls many of the biochemical processes in the plant can induce this attribute in the genome of the plantain, thus controlling multiple phenotypes like the phenotypic variants by the single *GTPase* single gene. Pleiotropy is a condition where a single genic mutation causes more than one phenotypic effect as observed in the multiple bunching phenomenon in plantain. In pleiotropy, one gene affects multiple characteristics (Ubi and Brisibe 2018).

7.12.2.4 Incomplete Penetrance

Penetrance is the proportion of individuals of a species with specific genotypes that show or manifest variable characteristics or phenotypes. It is a factor that influences the effects of particular genetic changes. Here, the genetic traits have reduced with incomplete penetrance, thus being expressed in only a part of the population. The inconsistency in multiple bunching phenomena in inflorescence polymorphic plantain can be attributed to incomplete penetrance of the genetic traits in the follower crop generations (Ubi and Brisibe 2018).

7.12.2.5 Transversional or Nonsynonymous Mutations

These types of mutations result in the transformation and conversion of amino acids from one form to another, thus significantly influencing protein expressivity. The changes in the study involve the conversion of purines to pyramidines and pyramidines to purines leading to the changes in genetic expressions. The changes in amino acid compositions in the genome of the plantain during the reproductive stage accounts for the changes from multiple bunch to single bunches in subsequent follower or ratoon crop generations. This is informed by the fact that in the single bunching phenotype, transition mutations in which there are no changes in amino acids compositions in the genome after mutation and hence no change in bunch phenotype (Ubi and Brisibe 2018).

7.12.2.6 Missense Mutations

These are changes in amino acids that results in phenotypic changes in gene expression. These have already been explained and are similar to transverse and nonsynonymous mutations (Ubi and Brisibe 2018).

7.13 Cytogenetics of Inflorescence Developmental Polymorphic Plantains

7.13.1 Cytology of Plantain Chromosome (AAB Genome)

Most cultivated *Musa* species are triploids (2n = 3x = 33). Being almost completely sterile, they develop fruits by parthenocarpy. The genome of cultivated varieties is derived from the diploid related to *Musa acuminata* and *M. balbisiana* with A and B genomes, respectively (Fig. 7.8).

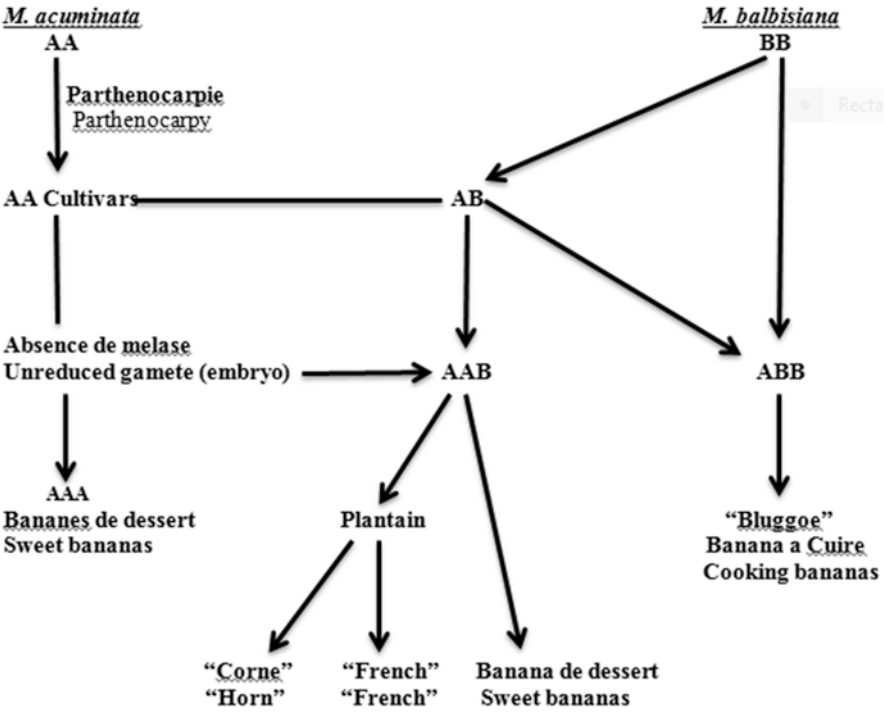

Fig. 7.8 Cytological development of AAB Genome of *Musa paradisiaca*. (Source*:* Gerda 1990)

The most important cultivars have characteristic genomic constitutions like dessert banana (AAA), East Africa highland banana (AAA), plantain (AAB) and cooking banana (ABB). (Simmonds 1959).

7.13.2 Fluorescence In Situ Hybridization in Plantain

The following conclusions can be made from the results of fluorescence in situ hybridization in multiple bunching plantain (*Musa paradisiaca*) accessions:

(a) Fluorescence in situ hybridization in the single-bunching plantain accession that served as the control in the study was observed to commence on a single chromosome 6 after dual multicolored fluorochrome (DAPI/DA) staining and rearrangements (Fig. 7.9a);
(b) The number of peduncles formed per inflorescence polymorphic plantain was found to be associated with the number of translocated chromosome(s) which hybridized to the DNA probe;

Fig. 7.9 (**a**) Fluorescence in situ hybridization of single bunching plantain showing triploid chromosomes with stable genome at 2n = 2x = 33, (**b**) fluorescence in situ hybridization of multiple bunching plantain showing diploid chromosomes with unstable genome at 2x = 2n = 22. (Source: Ubi and Brisibe 2018)

(c) The number of bunches per inflorescence polymorphic plantain was found to be associated with the maximum number of chromatids of chromosome pairs hybridizing with the DNA probe;
(d) Two pairs of hybridizing chromatids with the DNA probe were found to be associated with the double bunches;
(e) Three chromatids hybridizing with the DNA probe were found to be associated with triple bunches;
(f) Univalent chromosomes were found to be associated with the number of peduncles (Fig. 7.9b);
(g) Bivalent hybridized chromosomes were found to be associated with double bunching in plantain;
(h) Trivalent chromosomes were found to be associated with triple bunching in plantain.

7.13.3 *Genomic In Situ Hybridization (GISH) Study in* Musa *Species*

Racharak and Eiadthong (2007) in their studies with GISH on *Musa*, reported that it is feasible to detect the rate of development of univalent, bivalent and multivalent hybrids in a plantain population by comparison with ancestral genomes, and also the chiasma formation or recombination frequencies between homologous chromosomes. The great advantage is that, with this approach, it is possible to observe and identify the factors that induce irregular meiotic divisions, and how they affect plantain fertility.

The analysis of plantain plant behavior at meiosis was ascertained by use of genomic in situ hybridization of interspecific hybrids of *Musa* cultivated banana species (Ji and Chetelat 2003; Jin et al. 2006). These hybrids plantains were obtained from the cross between *M. acuminata* (2n = 2x = 22, AA) and *M. balbisiana* (2n = 2x = 22, BB). The interspecific triploid hybrids earlier referred to as Figure Pomme (2n = 3x = 33, AAB) and Praha (2n = 3x = 33, ABB) developed as multivalent, bivalent and univalent hybrids in both cultivars, with homologous bivalents seen in all chromosomes analyzed; and with all the multivalent hybrids (trivalent, tetravalent) showing homologous chromosomes. Research has shown that it is possible to re-engineer the recombination between the two genomes, A and B, which is very relevant in the domestication and improvement of interspecific plantain cultivars (Jeridi et al. 2011; Ji and Chetelat 2007).

The results of genomic in situ hybridization showed the following:

(a) No hybridization was observed between genomic and DNA segment probes in the single-bunching plantain accession, which consequently may be the reason for the persistence of the single bunching expression at every generation (Fig. 7.10a);
(b) The number of peduncles formed per inflorescence polymorphic plantain depended on the number of sites of hybridization between the target chromosomes and the genomic probe;
(c) The number of bunches per inflorescence polymorphic plantain accession depended on the number of chromatids of chromosomes hybridizing with the genomic probe (Fig. 7.10b);
(d) Two univalent chromosomes hybridizing in a T-shape manner was found to be associated with double bunches;
(e) Multiple bivalent chromosomes hybridizing in a random manner was found to be associated with the formation of triple bunches.

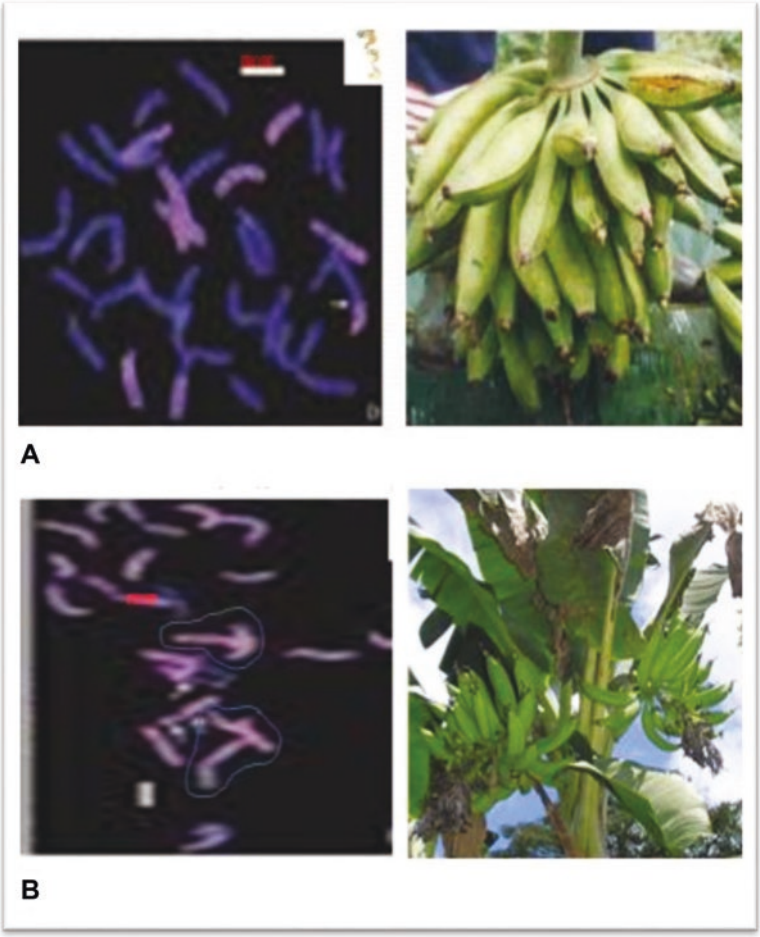

Fig. 7.10 Genomic in situ hybridization in a single bunching plantain (**a**) and in multiple bunching plantain (**b**). (Source: Ubi and Brisibe 2017)

7.14 Chromosomal Number and Architecture of Inflorescence Polymorphic Plantains

Considering the possibilities for ploidy variations in some plantain morphotypes, the ploidy level of all six cultivars were carefully re-evaluated in a second series of experiments in the plant, with first and second follower crops using DNA flow cytometric analysis to verify the mechanism of the crop-cycle dynamics in the inflorescence polymorphic plants. A total of 100 plantain plants (10 each for all 5 polymorphic variants during 2 crop cycles) were evaluated in the first and second ratoon crops, in which 95 were diploid and 5 triploid (Fig. 7.11), suggesting that the multiple-bunching plantains were mainly diploid and genetically unstable resulting

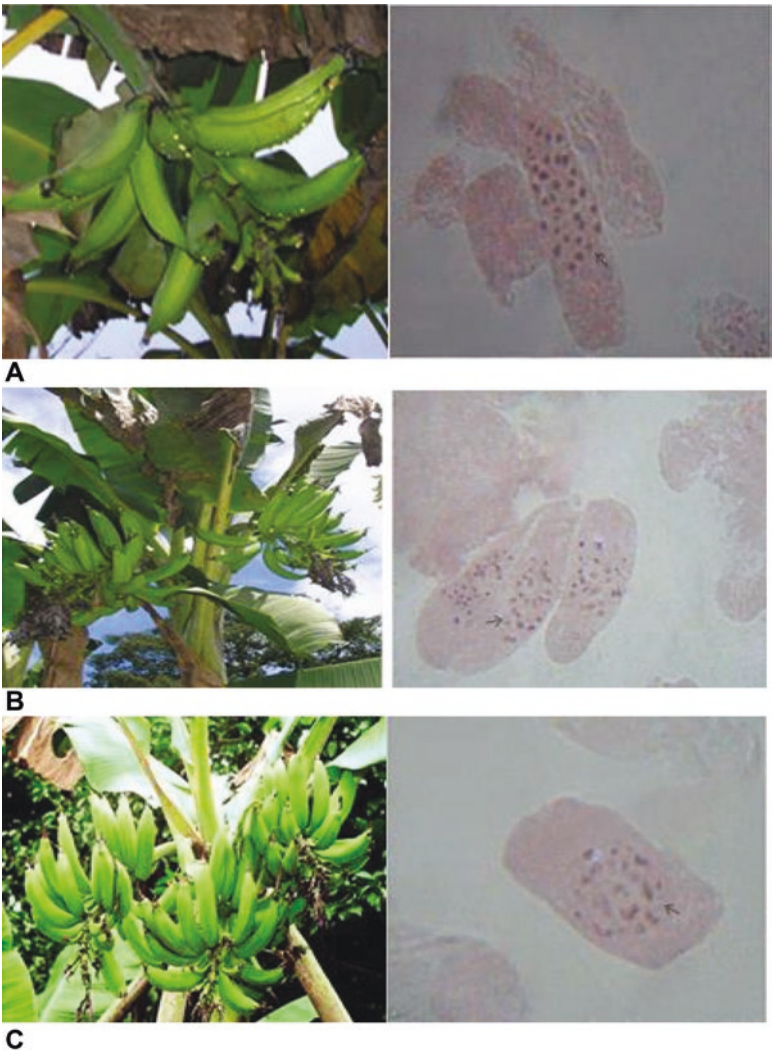

Fig. 7.11 (**a**) Stable triploid in single bunching plantain accession showing 2x = 2n = 33, (**b**) unstable diploid in multiple bunching plantain accessions showing 2x = 2n = 22, (**c**) unstable triple in multiple bunching plantain accessions showing 2x = 2n = 22. (Source: Brisibe and Ekanem 2019)

from mutations in some or all of the chromosomes. Concomitant with these variations in ploidy status, there were changes in the chromosome numbers from diploid (2n = 2x = 22) (Fig. 7.11a) to the more stable triploid (2n =3x =33) (Fig. 7.11b), usually seen as a natural attribute of all plantain landraces. Thus, it can be speculated that the ploidy changes identified may result from different amounts of genetic changes that may have taken place as the number of both bunches and crop cycles increased. Collectively, these observations tend to suggest the presence of a high

incidence of genetic instability in the inflorescence dichotomous plantains early in the crop cycle. However, these genetic changes are favorably more disposed towards achieving the more stable triploid status in later cropping cycles of the follower crops, especially as the number of bunches obtained on a single plant showed reduced bunches instead of an increasing number of bunches. Based on this phenomena and observations, it therefore created a heightened need of major horticultural significance, for the additional studies to be designed which would help to examine and unveil the genetic and environmental basis of these ploidy level changes since cultivars with a higher number of fruit bunches appeared to be more unstable than those with the conventional single bunch.

7.15 Bioinformatics Studies in Multiple Bunching Plantain Accessions

The *GTPase* gene of the complex *leaf tissue* gene in multiple bunching plantain accessions were sequenced after amplification of the gene. The chromatograms containing the sequences were further analyzed using Chromas software to obtain the individual sequences. The sequences obtained were further analyzed using Bioedit software, molecular evolutionary and genetic analysis (MEGA X) software, and some online programs like the Expasy, Genscan, Nsopma and Phyre and Phyre to obtain the properties of the gene, SNPs, mutations types, phylogenetic tree relationship and their tertiary protein structures.

7.15.1 Time of Divergence and Evolutionary Relationship Among Inflorescence Polymorphic and the Single Bunching Plantain Accessions

As shown in the phylogenetic tree (Fig. 7.12), the plantain accession with double bunches borne on one peduncle was the nearest neighbor to the single-bunching accession in terms of evolutionary sequence. The time of genetic divergence for the single-bunching plantain was zero years (0 MYA, million years ago).

The accessions with double bunches borne on one peduncle and double bunches on two peduncles were very close in terms of their evolutionary sequence and mutation with 99% similarity that showed a genetic divergence time of 10 MYA.

The percentage similarity in evolutionary changes between the plantain accession with triple bunches on one peduncle and the double bunches in two peduncles is 97% with a relative genetic divergence time of 12 MYA.

The percentage similarity in evolutionary changes between the triple bunches on one peduncle and triple bunches on two peduncles is 91% showing a slight variation in mutation. Time of genetic divergence of the gene in this inflorescence polymorphic plantain accession is 20 MYA.

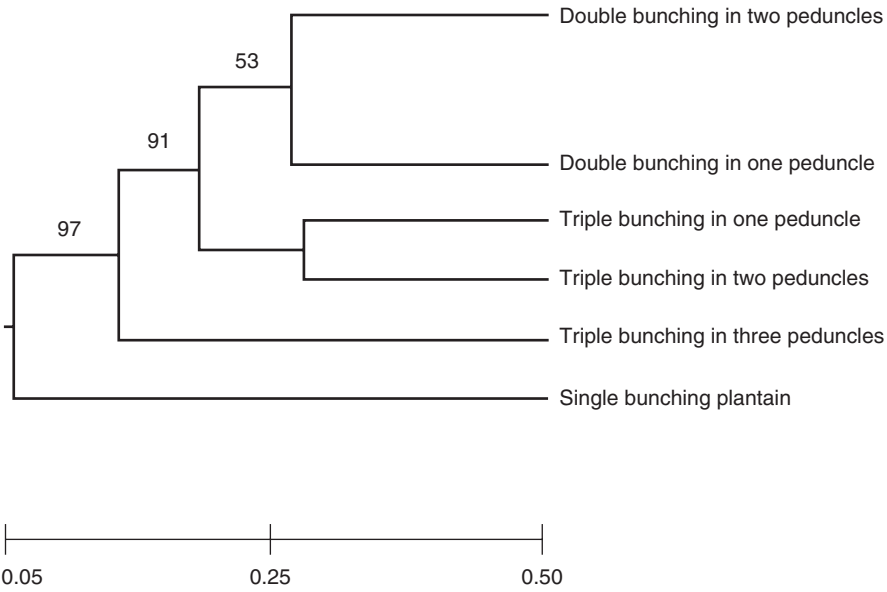

Fig. 7.12 Phylogenetic tree showing evolutionary relationships and time of divergence of the *GTPase* protein binding genes among the single and multiple-bunching plantain accessions. (Source: Ubi et al. 2017a, b)

The percentage similarity in evolutionary changes between the triple bunches on three peduncles and the other triple-bunching polymorphic types is 53% showing that significant changes in evolutionary sequence and mutation have taken place. The average time of genetic divergence for this inflorescence polymorphic plantain accession is 15 MYA.

Multiple sequence alignment data are indicative of the differences in chromosome numbers and ploidy levels among the different cultivars evaluated and provided some insights into the polymorphism phenomena detected. However, the cascade of reactions in the genome which triggered these phenotypic variations in plantain still remains unclear. Thus, to have a better understanding of the molecular mechanisms underlying this highly unstable genetic attribute, a possible strategic study approach should involve nucleotide diversity studies and single nucleotide polymorphisms (SNPs) exploration and identification in inflorescence dichotomous plantains. This initiative could find/identify diverged and conserve regions in the gene sequences associated with floral development and which are utilized in the design of primers that are further used in the polymerase chain reaction (PCR), thus annealing the amplicons to only a single DNA target (Zwierzykowski et al. 2008).

Multiple sequence alignments created from pairwise alignments used to select the highly conserved variation-enriched regions are presented in Fig. 7.13. Two distinct attributes were identified. First, there was a major nucleotide deletion in the inflorescence dichotomous cultivars (Fig. 7.13). Second, there was a high level of nucleotide diversity in the genome of the inflorescence dichotomous cultivars,

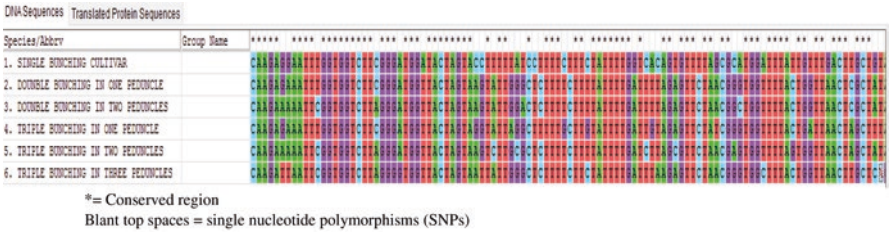

Fig. 7.13 Multiple sequence alignment of inflorescence polymorphic plantain showing single nucleotide polymorphisms and conserved regions. (Source: Ubi and Brisibe 2018)
* = Conserved region
Blant top spaces = single nucleotide polymorphisms (SNPs)

Table 7.6 Base pairs of nucleotide sequences and number of amino acids of *GTPase* gene of the *leaf tissue* complex gene of inflorescence polymorphic and single-bunching plantain accessions

S/N	Accessions	Number of nucleotide (bp)	Number of amino acid	Genome
1	Single bunch control	1185	221	AAB
2	Double bunches in one peduncle	3478	83	AAB
3	Double bunches in two peduncles	3486	86	AAB
4	Triple bunches in one peduncle	3890	105	AAB
5	Tripe bunches in two peduncles	3864	105	AAB
6	Triple bunches in three peduncles	3862	105	AAB

Source: Ubi and Brisibe (2018)

which are represented as nucleotide swaps that may have resulted from duplicated loci or allele separation from the ancestors at the time of divergence from the single-bunching cultivar (Fig. 7.12). Descriptive details for the single nucleotide polymorphic loci, nucleotide substitutions types and amino acid swaps in the genomic sequences of the different cultivars are shown in Table 7.6. As expected, the single-bunching plantain cultivar was genetically stable as it possessed the least number of nucleotide changes with no associated nucleotide substitutions. Hence, all the variations in the base pair in the aligned sequences involved only a change in position of the last base nucleotide (for a three base codon) resulting in a swap of amino acids from guanine to adenine and adenine to guanine, without the prediction of any concomitant changes in the expressed proteins (Table 7.6).

Of particular interest and significance is that this aspect of observations has revealed that transitional and synonymous point mutations in single nucleotide polymorphisms (that involving a change from purine to purine and pyrimidine to pyrimidine or leucine to leucine and lysine to lysine and vice versa) were only observed and peculiar to the single-bunching plantain cultivar. This obviously, is an indication that silent mutation (with no changes on amino acid sequence),

Table 7.7 Transitions, transversions, synonymous and non-synonymous mutations in the *GTPase* gene of the *leaf tissue* gene complex of inflorescence polymorphic accessions of plantain

Plantain accession	Number of SNP sites	Nucleotide substitution	Transitions/ transversions	Mutation type
SBC	55	Pu-Pu	Transition	Synonymous
DB1P	82	Py-Py	Transversion	Nonsynonymous
DB2P	77	Pu-Py	Transversion	Nonsynonymous
TB1P	81	Py-Pu	Transversion	Nonsynonymous
TB2P	79	Pu-Py	Transversion	Nonsynonymous
TB3P	86	Pu-Py	Transversion	Nonsynonymous

Source: Ubi and Brisibe (2018)
SNP single nucleotide polymorphism, *Pu* purines, *Py* pyrimidines

consequently does not lead to an alteration in the expression and function of the *GTPase* complex *leaf tissue* gene. This provides experimental evidence and the possibility that the absence of any changes in amino acid sequence may have accounted for the genetic stability that was observed in all replicates of the single-bunching plantain cultivar. On the contrary, a number of transversions and nonsynonymous single nucleotide changes were detected in the coding regions of the *GTPase* complex *leaf tissue* gene (Table 7.7), depicting that the amino acid substitutions resulted in the alternative receptor isoforms. These changes in amino acid types can be speculated to have contributed to and be responsible for the bunch variability that was detected in the inflorescence dichotomous cultivars.

However, unlike in the single-bunching cultivar, the basic nucleotide changes detected in the inflorescence polymorphic phenotypes were precisely from purine to pyrimidine and pyrimidine to purine while some of the typical amino acid changes in the different accessions included glycine to valine in DB1P, leucine to proline in DB2P, valine to lysine in TB1P, leucine to glycine in TB2P and glycine to valine in TB3P, respectively (Table 7.7). These different change manifestations resulting in nucleotide substitutions and changes in the types and positions of amino acids equally suggest highly complex processes that can be associated with alterations in both gene function and expression. It is speculative from these details, therefore, that the high mutation rates characteristic of both nucleotide base pairs and amino acids could have led to the high genetic instability detected, which may have developed as a consequence of incomplete penetrance or pleiotropy and may equally have contributed to the presence of multiple genetic variants within the *Musa* population evaluated.

Table 7.7 shows that transitional and synonymous point mutations in single nucleotide polymorphisms (SNPs) were only peculiar to the single-bunching plantain accession (that is change of purine to purine and pyrimidine to pyrimidine/ leucine to leucine and lysine to lysine) indicating only a change in position of the same amino acid, which does not alter the gene expression and function.

All forms of inflorescence polymorphic plantain accessions were found to undergo transversional and nonsynonymous point mutations in their single nucleotide polymorphisms (SNPs). Changes observed showed purine to pyrimidine and

pyrimidine to purine/glycine to valine and lysine to proline. These changes in types and position of amino acids are responsible for the change and alterations in gene functions and expressions leading to genetic instability.

This was thus probably seen to be responsible for the high genetic instability in the inflorescence polymorphic plantain accessions and instability of the gene expression for the yield traits and thus responsible for the nonpersistence of inflorescence polymorphism in the False Horn plantain accessions.

7.16 Tertiary Protein Structures of Inflorescence Polymorphic Plantain

The tertiary protein structure characteristics for the inflorescence polymorphic plantain variants were determined using the protein homology Y recognition engine (PHYRE) online interactive tool and the protein sequences. The tertiary protein structure reflected the bunch phenotypes corresponding to the extended strand components (Ubi and Brisibe 2017). These are discussed relative to the Fig. 7.14a–c shown below.

The protein structures exhibited by the different inflorescence polymorphic plantains and single-bunching plantain accessions can be summarized as follows:

(a) Only 1 extended strand was observed in the single-bunching accession that corresponded to the single peduncle (Fig. 7.14a);
(b) In DB1P, 2 extended strands were joined at one end to produce one peduncle with 2 bunches (Fig. 7.14b);
(c) In DB2P, 2 separate extended strands each corresponding to a peduncle with 1 bunch;
(d) In TB1P, 3 extended strands that were joined at one end corresponding to 1 peduncle with 3 bunches (Fig. 7.14c);
(e) In TB2P, 2 extended strands joined at one end to form a peduncle with 2 bunches and another separate extended strand corresponding to a peduncle with a single bunch;
(f) In TB3P, 3 separate extended strands each corresponding to a peduncle with a single bunch.

7.17 Conclusions and Prospects

Diversity studies of elite plantain cultivars in Nigeria have provided baseline information and created awareness on the genetic, species and ecological diversity of elite plantain (*Musa paradisiaca*) cultivars in Cross River state. This represents a significant step in the right direction toward the collective struggle to eradicate poverty, hunger and unemployment of the citizenry. This chapter provides baseline information needed for urgent intervention in the preservation, conservation,

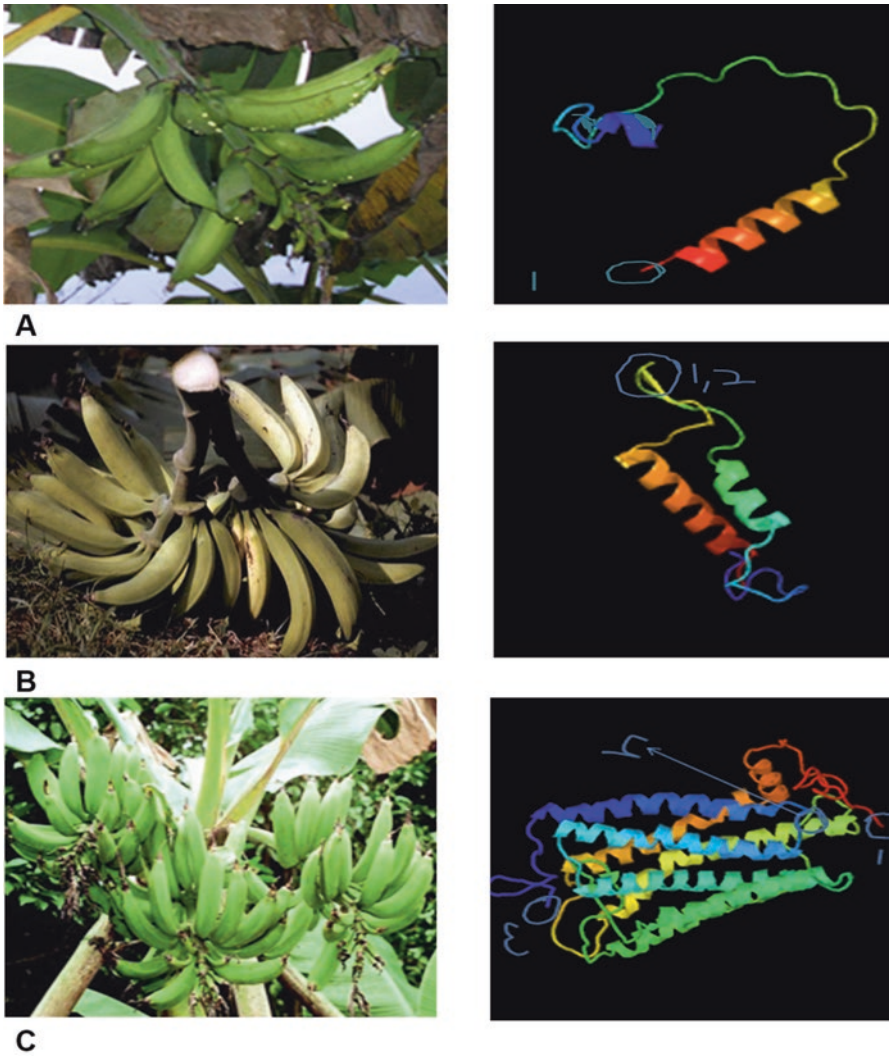

Fig. 7.14 (**a**) Tertiary protein structure for single bunching plantain showing 1 extended strand corresponding to single bunching, (**b**) tertiary protein structure for double bunching plantain showing 2 extended strands corresponding to double bunching, (**c**) tertiary protein structures for triple bunching plantain showing 3 extended strands corresponding to triple bunching. (Source: Ubi and Brisibe 2017)

improvement and management of plantain genetic resources. Plantain is an important cash crop in Nigeria and forms the mainstay of most rural economy in the state and country at large.

With the teeming population and the upsurge in rural-to-urban migration, youth restiveness, high unemployment rate, high rate of poverty and increased crime rate among Cross Riverians, it is imperative for a holistic and collective approach to be

adopted for the improvement, conservation, production, management and utilization of the elite plantain cultivars for the attainment of sustainable development drive for contemporary Cross River state.

It has also been revealed that current breeding techniques such as micropropagation through somatic embryogenesis, in vitro propagation, temporary immersion systems (bioreactors), haploid production, induced mutation breeding, cryopreservation and the use of virus free suckers can be widely utilized and applied in the development and increased production of plantain for food security. This will probably offer significant relief to the resource-poor farmers who are struggling against these traditional obstacles and barriers to plantain production, especially in developing country like Nigeria.

The study on the molecular, cytogenetic and phylogenetic characteristics of inflorescence developmental polymorphism in plantain is an important approach toward unveiling the probable causes and reason(s) for the nonpersistence of this yield attribute in plantain, a phenomenon that has generated so much interest among plant breeders and farmers. This study revealed that the phenomenon of inflorescence developmental polymorphism in plantain accessions occurs mostly among the False Horn varieties. The variability in the phenotypic expression of this trait was highly contributed to by the first principal component.

From the molecular, cytogenetic and phylogenetic characteristics of inflorescence polymorphic plantain accession unveiled by this study, it could be concluded that transversional, non-synonymous point mutations of the highly unstable *GTPase* gene of the *leaf tissue* gene complex are responsible for the nonpersistence of inflorescence developmental polymorphism in False Horn plantain (AAB group). This review chapter has revealed the following thrusts:

Some of the molecular, cytogenetic and phylogenetic basis underlying the dichotomous phenomenon in plantain revealed in this chapter shows that molecular and genetic data and information are available on multiple-bunching plantain accessions. The basic types of mutations associated with nonpersistence of inflorescence polymorphism among variant accessions in the field are discussed.

Chromosomal translocations also occur in inflorescence polymorphic plantain accessions during in situ hybridization. The meiotic behavior of plantain polyploids was associated with unstable genetic composition. Fluorescence and genomic in situ hybridization techniques as cytogenetic tools can be used to reveal the cytogenetic mechanisms in inflorescence polymorphic plantain accessions. Molecular and phylogenetic characterization studies can be useful in promoting and sustaining biodiversity in plantain species.

Important prospects of future research and utilizations include the production of multiple bunching plantain contributes positively to food security, especially in the current global pandemic era. Conservation efforts geared towards the preservation of multiple bunching plantain germplasm in gene banks can be very promising towards the crop future production, management and utilization. In addition, more research is needed to reveal the underlying factors necessitating the inconsistencies associated with inflorescence developmental polymorphism in plantains. Biotechnological approaches should be adopted in the production, management and

conservation of plantain germplasm. Governments should encourage multiple bunching plantain farming by establishing mandated institutions with a view to ensuring food security, creating employment and means of livelihood for her teeming populace as the world look forward to developing post COVID-19 economy recovery strategies.

Appendix I: Research Institutes Relevant to Plantain

Institution name and location	Website
International Institute of Tropical Agriculture IITA, Moore Plantation, Ibadan, Nigeria	www.cgiar.iita.org
Farm Focus International FFI, Calabar South, Cross River State, Nigeria	www.farmfocusinternational.org
University of Calabar, Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria	www.unical.edu.org
Australian Plant DNA Bank (APDB), Centre for Plant Conservation Genetics, Southern Cross University, Lismore, NSW, Australia	http://www.dnabank.com.au
Botanic Garden and Botanic Museum (BGBM) DNA Bank, Berlin, Germany	http://www.bgbm.org/bgbm/research/dna/
DNA Bank Brazilian Flora Species, Rio de Janeiro Botanic Garden, Brazil	http://www.jbrj.gov.br/pesquisa/div_molecular/bancodna/index.htm
DNA Bank at Kirstenbosch, South African National Biodiversity Institute, Kirstenbosch, South Africa	http://www.nbi.ac.za/research/dnabank.htm
International Rice Research Institute (IRRI), DNA Bank, Philippines	http://www.irri.org/GRC/GRChome/Home.htm
Missouri Botanic Garden DNA Bank, (MBGDB) St Louis, MO, USA	http://www.mobot.org/MOBOT/research/diversity/dna_banking.htm
National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India	http://www.nbpgr.ernet.in/
National Herbarium Netherlands DNA Bank (NHNDB), Netherlands	http://www.nationalherbarioum.nl/taskforcemolecular/dna_bank.htm
National Institute of Agrobiological Science (NIAS) DNA Bank, Tsukuba, Ibaraki, Japan	http://www.dna.affre.go.jp/
Plant DNA Bank Korea (PDBK), Graduate School of Biotechnology, Korea University, Seoul, Korea	http://www.pdbk.korea.ac.kr/index.asp
Royal Botanic Garden Edinburgh DNA Bank, Edinburgh, Scotland	http://www.rbge.org.uk/rbge/web/science/research/
Royal Botanic Garden Kew DNA bank, Richmond, England	http://www.rbgkew.org.uk/data/dnaBank/
TCD DNA Bank, Department of Botany, School of Natural Sciences, Trinity College, Ireland	http://www.dnabank.bot.ted.ie
Tropical Plant DNA Bank, Fairchild Tropical Botanical Garden and Florida International University, FL, USA	http://www.ftg.org/research
International Institute for Tropical Agriculture, Nairobi, Kenya	www.cgiar.iita.org

Appendix II: Some Genetic Resources of Plantain, Cultivation Area Genome Group and Main Characteristics

Cultivar	Cultivation location			Genomic group	Main characteristics				
					Finger size/ shape	Bunch phenotype	Finger cross section	Unripe Finger skin color	Unripe Pulp color
Elite plantain cultivars	Latitude (N)	Longitude (E)	Elevation (m)	Genome/ type	Big/curve	False horn	Triangular	Dark green	Creamy
Enugu black	06° 02.84′	08° 41. 10′	210	False horn/ AAB	Big/curve	False horn	Quadrilateral	Pale green	Milky white
Ebi Egome	05° 56.54′	08° 50. 45′	132	False horn/ AAB	Medium/ curve	French type	Pentagonal	Red wine	Milky white
Ogoni red	06° 54.58′	09° 17. 79′	178	French/AAB	Medium/flat	False horn	Quadrilateral	Brown	Creamy
Kigwa Brown	06° 48.62′	09° 15. 30′	183	False horn/ AAB	Medium/flat	True horn	Triangular	Pale green	Creamy
Ejorgom	06° 30.72′	09° 10. 68′	119	True horn/ AAB	Small/curve	French type	Pentagonal	Pale green	Milky white
Bakpri (dwarf mutant)	04° 97.78′	08° 36. 01′	54	French/AAB	Medium/ curve	False horn	Quadrilateral	Pale green	Creamy
Owomoh	05° 55.88′	08° 26. 39′	175	True horn/ AAB	Medium/flat	False horn	Quadrilateral	Dark green	Creamy
Kainjen	05° 58.20′	08° 63. 52′	181	False horn/ AAB	Small/curve	French type	Triangular	Dark green	Milky white

Ikpobata (cooking banana)	06° 28.43′	09° 08. 85′	97	French/ABB	Big/flat	False horn	Triangular	Pale green	Milky white
Mgbeghe	05° 38.71′	08° 46. 02′	119	False horn/ AAB	Big/flat	False horn	Quadrilateral	Pale green	Milky white
Kenkwa	06° 04.45′	08° 54. 77′	130	False horn/ AAB	Medium/ curve	French type	Quadrilateral	Dark green	Creamy
Uhom	05° 42.19′	08° 03. 23′	56	False horn/ AAB	Medium/ curve	French type	Triangular	Pale green	Creamy
Ekunkwam	06° 33.46′	08° 52. 29′	110	French/AAB	Medium/ curve	True horn	Pentagonal	Pale green	Milky white
Ingwam	06° 39.99′	08° 51. 61′	92	French/AAB					

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