# Induced genetic diversity in banana

Suprasanna , P

Springer
2019

Suprasanna , P , Ghag , S , Ganapathi , T R & Jain , S M 2019 , Induced genetic diversity in banana . in D Nandwani (ed.) , Genetic Diversity in Horticultural Plants . Sustainable Development and Biodiversity , vol. 22 , Springer , pp. 273-297 . https://doi.org/10.1007/978-3-319-96454-6

http://hdl.handle.net/10138/323962
https://doi.org/10.1007/978-3-319-96454-6

acceptedVersion

Sustainable Development and Biodiversity 22

Dilip Nandwani *Editor*

# Genetic Diversity in Horticultural Plants

Springer

Dilip Nandwani
Editor

# Genetic Diversity in Horticultural Plants

Springer

# Chapter 10
# Induced Genetic Diversity in Banana


**Suprasanna Penna, Siddhesh B. Ghag, T. R. Ganapathi and S. Mohan Jain**

**Abstract** Banana and plantains are one of the important fruit crops grown extensively in the tropical and subtropical regions of the world. The world production of banana is 145 million tons of which only a few million tons is exported, which means that most production is primarily for local consumption. The banana cultivars are derived from two diploid wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome). Majority of the edible banana cultivars are propagated vegetatively, and hence, the improvement of banana through conventional breeding methods is difficult. Attempts have been made to improve banana by inducing genetic variability by using both physical and chemical mutagens and exploiting the somaclonal variation a few varieties have been released for cultivation. Transgenic approach has also been used to incorporate the desirable traits into banana. Recent advances in genomics and the availability of genome sequence of both *Musa acuminata* and *Musa balbisiana* helps in the improvement of this fruit crop. Also the recent reports of genome editing through CRISPR-CAS9 will aid in speeding up the banana improvement programmes in the near future. This review summarizes the various advances made in inducing genetic diversity in banana.

**Keywords** Banana · Genetic Diversity · Mutation induction · Somaclonal variation transgenics

S. Penna · T. R. Ganapathi
Nuclear Agriculture & Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India
e-mail: penna888@yahoo.com

S. B. Ghag
School of Biological Sciences, UM-DAE Centre for Excellence in Basic Sciences, Kalina, Mumbai 400098, India

S. M. Jain (✉)
Department of Agricultural Sciences, University of Helsinki, PL-27, Helsinki, Finland
e-mail: mohan.jain@helsinki.fi



D. Nandwani (ed.), *Genetic Diversity in Horticultural Plants*,
Sustainable Development and Biodiversity 22,
https://doi.org/10.1007/978-3-319-96454-6_10

## 10.1 Introduction

Banana and plantain (*Musa* sp.) belong to a group of edible, vegetatively propagated, monocotyledonous and herbaceous species, belonging to the Eumusa section of the genus Musa, family Musaceae and order Zingiberales (Gill 1988). Bananas are the fourth important food crop after major cereals and are consumed locally in many developing countries (FAO 2002). Banana production is around 145 million tons of which only a few million tons is exported, which means that most production is primarily for local consumption (FAO 2015). This poor man's fruit contributes very much to food and nutritional security. The fruit has significantly shares (25%) the total carbohydrate requirements of African countries (Robinson 1996). Banana fruit has majorly carbohydrates (35%) followed by fibre (6–7%), low protein and fat (1–2%), and major elements such as potassium, magnesium, phosphorus, calcium, iron, and vitamins A, B6 and C (Robinson 1996). Normally, bananas are consumed in ripe and starchy form, or boiled or cooked in different traditional cooking (Frison and Sharrock 1998). It is also an important source for making beer, wine and other products (Stover and Simmonds 1987). There are also a number of other food products including jam, juice and squashes, banana chips or crisps, sweet banana figs, banana flour, banana powder and starch (Padam et al. 2014). Banana fruit also has several valuable bioactive compounds such as phenolic compounds, carotenoids, biogenic amines and phytosterols (Singh et al. 2016), and they have antioxidant property that serves as a defence arsenal against oxidative stress.

The genus Musa has members which are seeded (wild) and no seeded or parthenocarpic edible types (Ortiz 1995). Most cultivars are derived from two diploid wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) (Osuji et al. 1997). Edible clones are classified based on the relative contribution of *M. acuminata* and *M. balbisiana* (Simmonds and Shepherd 1955). Around 30–40 species are present under the genus *Musa* (Simmonds 1987), and all the wild varieties are diploids with 14, 18, 20 or 22 chromosomes. On the basis of number of chromosomes, and floral arrangement in inflorescence, this genus is classified in five sections, namely Australiamusa, Callimusa, Emusa, Ingentimusa and Rhodochlamys. These include the wild varieties, i.e. seeded, marketed edible bananas, i.e. non-seeded and parthenocarpic ones (Ortiz et al. 1995). Section *Callimusa* and *Rhodochlamys* are cultivated as ornamentals and do not produce fruits, whereas *Australimusa* members are highly infertile (Jarret et al. 1992) and comprise *M. textilis Nees* (Abaca) cultivated for fibre production (Cheesman 1949), and the seedless edible *Musa Fehi* (Fe'i bananas) contain higher vitamin A content.

*Eumusa* (true bananas) is the largest of all, and the inflorescence is pendent or semi-pendent type. It includes 13–15 species of bananas including the ancestors of triploid bananas; *Musa acuminata* (A genome) and *balbisiana* (B genome). This is the most diversified, ancient and widely distributed section of genus *Musa*. The best examples of this group are the commercially cultivated Cavendish bananas like Grand Nain (AAA), Giant Cavendish (AAA), Williams (AAA), Robusta (AAA), Dwarf Brazilian (AAB), etc. It also contains *M. schizocarpa* (S genome). These

present-day cultivated edible bananas have chromosome numbers of 22, 33, 44 with basic number as $n = x = 11$ (Heslop-Harrison and Schwarzacher 2007). These are hybrids of A and B genome and are a result of natural crosses between two wild species, *Musa acuminata* (AA genome) and *Musa balbisiana* (BB genome) (Osuji et al. 1997). The diploid bananas grown today AA, BB or AB; however, the most prevalent and commercially grown most important bananas are the triploids, while a few tetraploids are available in nature or they are artificially developed by employing the breeding methods (Patel et al. 2016; Menon 2016).

Banana, being triploid and vegetatively propagated, has inherent constraints of low seed set and germination rates. Conventional breeding of banana has limitations of ploidy in relation to hybridisation, morphology and yield, parthenocarpy and sterility, and long time-consuming steps of hybrid development (Vuylsteke et al. 1993; Oselebe et al. 2006; Creste et al. 2004). Banana production is challenged by both biotic and abiotic stresses. Considerable attention has been paid on biotic stress factors (Kotari et al. 2016); however, research on abiotic stresses has lagged behind, and only in the past 5–10 years, there has been increasing research in this direction (Ravi and Vaganan 2016).

Banana cultivation is challenged by plant's vulnerability to pests and diseases. The most significant ones include the black Sigatoka caused by *Mycosphaerella fijiensis* Morelet, Panama disease (*Fusarium oxisporum*, race 1 and 4), bacteria *Pseudomonas solanacearum*, viruses such as the banana bunchy top virus (BBTV), cucumber mosaic virus (CMV), nematodes *Radopholus similis* and weevils *Cosmopolites sordidus*. Both conventional and biotechnological approaches are being used in improving disease/pest resistance and quality. Cavendish-type bananas exhibit susceptibility to most of these diseases. Wild species of *Musa* are known to have resistance to black Sigatoka, for example, *M. acuminata* ssp. Burmannica, ssp. Malaccensis and ssp. siamea, and diploid AA cultivars.

Most of the bananas exhibit susceptibility to abiotic stresses like drought, salinity and extreme temperature. It has been shown that different genomic groups exhibit considerable genetic variability for abiotic stress tolerance (Hu et al. 2017). The 'ABB' banana genotypes are tolerant to drought and other abiotic stresses, and hence, are a good genetic resource for use in breeding attempts to improve abiotic stress tolerance (Ravi et al. 2013). According to Simmonds (1966), bananas are grown in four different climatic zones. These include climates where bananas experience little or no seasonal growth, without much need for irrigation, climates where bananas experience seasonal drought or a combined drought and low temperature, marginal climates where bananas survive and bear fruit only under favourable conditions of soil, irrigation and genotype management, and climates where bananas are grown under irrigation throughout the growing season.

Musa gene pool consisting of important wild species can be exploited for genetic improvement of certain traits which include tolerance to cold (*Musa sikkimensis* Kurz, Musa basjoo P. F. (B.) von Siebold ex Iinuma, Musa thomsonii (King ex Schumann) Cowan and Cowan), waterlogging (*Musa itinerans* Cheesman) and drought (*Musa balbisiana* Colla, Musa nagensium Prain) (INIBAP 2006; Oselebe et al. 2006). Several banana-growing countries have crop improvement programmes to

improve local germplasm for biotic and abiotic stress tolerance and improved fruit quality. A global initiative, International Musa Testing Program (IMTP; http://www.promusa.org/IMTP), has become significant for evaluating and release (through trials across counties and locations) of different cultivars, landraces, selections and hybrids for agronomy, pests and diseases, post-harvest traits, for adoption to different geographical zones and local conditions (Orjeda 2000). Molecular characterization of *Musa* genetic diversity assumes significance for exploring genetic markers in *Musa* improvement programmes (Till et al. 2010). In a first report, these authors found Ecotilling method to be effective for the discovery of nucleotide polymorphisms in diploid and polyploid accessions of Musa. The results revealed more than 800 novel alleles in 80 accessions suggesting that the method of Ecotilling can be useful for investigating into genetic polymorphisms in banana. The meristematic tissues of banana are treated with a mutagenic agent, and then plants are regenerated. The regenerated plants are multiplied, and the meristematic cells are successively isolated and bisected to obtain tissues devoid of chimeras. The problem of chimeras can be eliminated by optimizing protocol for mutagenesis or using single-cell cultures for mutagenesis. However, development of cell suspension cultures and regeneration of plant from a single cell is very established in banana. Above all, since banana is vegetatively propagated and do not set seeds, the induced mutations will be fixed in the clonal plants (Jankowicz-Cieslak et al. 2012).

Banana is vegetatively propagated, and hence, there is a greater need to induce additional genetic variability to enable selection for improved agronomic and quality traits. Biotechnological methods such as organogenesis, plant regeneration, mutagenesis, transgenic methods and molecular markers have been deployed (Jain and Swennen 2004). In banana, in vitro shoot-tip culture is most practised for micropropagation of commercial and novel genotypes. It has been very well established that plant developed through micropropagation grow better than those grown via suckers and have uniform growth (Robinson 1996). In vitro culture also guarantees safe collection, exchange and conservation of germplasm required for identification of breeding traits, and facilitates dissemination and propagation of newly selected cultivars or hybrids. The method of meristem culture is also very useful for the production of disease-free planting material and for preservation of novel banana genotypes (Cronauer and Krikorian 1984; Hwang et al. 1994; Helliot et al. 2002).

Somatic embryogenesis and high-frequency plant regeneration from commercial varieties of banana are prerequisites for realizing the potential for large-scale production of planting material and cellular and molecular approaches for crop improvement. Although different explants like scalps (proliferating meristems), rhizomes, leaf bases, immature zygotic embryos and young male flowers can be used for induction of somatic embryogenesis, immature male inflorescences and proliferating meristem sections have been successfully employed to initiate embryogenic cultures of several banana and plantain cultivars (Escalant et al. 1994; Navarro et al. 1997; Ganapathi et al. 1999; Suprasanna et al. 2001; Kulkarni et al. 2006; Sidha et al. 2011). Embryogenic cell suspensions (ECS) have become useful for use in genetic manipulation using different biotechnological tools, and relative success in genetic engineering of bananas is often dependent on this ECS system (Ghag and Ganapathi

2017). The method involves initiation of embryogenic callus, induction of cell suspension and assessment of regeneration ability. There has been good progress in the past 5–10 years, and for a wide range of banana genotypes, embryogenic callus can be induced and embryogenic cell suspensions (ECS) can be routinely established from embryogenic calluses of different cultivars of banana (Strosse et al. 2006).

## 10.2 Mutagenesis and Induction of Novel Genetic Variation

In vitro mutagenesis followed by in vitro selection is a very useful method as different cell, organ and tissue cultures can be mutagenized, uniform mutagen treatment can be given, large number of samples can be handled in a short span of time, large mutant population can be raised to separate chimeras, and it has options for including selection agents in culture media for in vitro selection (Van Harten 1998). In vitro mutagenesis is an effective method for the induction, and the selection of somatic mutations and increased mutant recovery may be possible through lower somatic competition by modifying culture conditions (Suprasanna et al. 2012). Plant growth regulators, and in particular a cytokinin, can increase the recovery rate of mutated cells. Hence, the combined use of mutation induction and in vitro technology is more efficient because it speeds up the production of mutants as a result of an increased propagation rate and a greater number of in vitro generations (Morpurgo et al. 1997).

Mutation induction using physical and chemical mutagens has been practised for generating useful mutations in banana. Physical mutagens like gamma rays have high and uniform penetration of multicellular system. Gamma irradiation results in small deletions (1–10 bp) while neutrons cause 300 bp to 12 kbp deletions, and chemical mutagens result in point mutations, mainly G/C-to-A/T transitions (Morita et al. 2009). On the other hand, ion beams have high linear energy transfer (LET) ranging from 22.5 to 4000 keV $\mu m^{-1}$ compared to 0.2–2 keV $\mu m^{-1}$ LET of $\gamma$-rays and X-rays (Ryuto et al. 2008). Heavy-ion-beam (HIB) irradiation is shown to be superior for mutation breeding as higher rate of mutations can be obtained at low doses (Hirano et al. 2015). Ion-beam techniques have also been used as they frequently produce large DNA alterations such as inversions, translocations and large deletions rather than point mutations. Reyes-Borja et al. (2007) reported use of ion-beam irradiation for mutation breeding in banana in selecting lines tolerant to black Sigatoka. The effect of irradiation doses on the regeneration of plantlets was investigated in Japan, and the variation in black Sigatoka response under field condition was evaluated in Ecuador.

For mutagenesis, both types of in vivo and in vitro explants are used in banana (Jain et al. 2011). During the early sixties, seeds and suckers of *Musa balbisiana* were used for gamma-ray mutagenesis, and it was observed that rate of seed germination and seedling survival were affected by the radiation dose (Stotzky et al. 1964). In a further study, Menendez (1973) treated seeds of diploid *Musa acuminata* with ethyl methanesulphonate (EMS). Irradiation of suckers prior to isolation of shoot-tip explants and in vitro culture gave a low yield of mutagenized material for further

screening (De Guzman et al. 1976). Irradiation of suckers prior to tissue culture initiation is not effective and can only yield low number of plantlets in the M1V1 generation because of the large sucker size, and their number is often critical for managing either physical or chemical mutagenesis. Karmarkar et al. (2001) compared radiosensitivity of different in vivo and in vitro planting materials and observed a decrease in per cent survival of irradiated material with increasing irradiation dose. In terms of radiosensitivity, hardened plants were most sensitive followed by suckers and in vitro shoots. Multiple shoot cultures of banana variety 'Giant Cavendish' were gamma irradiated at different doses (5, 10 and 30 Gy), and the irradiated population was field evaluated for different morphological and yield contributing traits. From one of the mutant lines (a 10 Gy mutant), dwarf mutant was identified (Fig. 1) and multiplied for further evaluation (Ganapathi et al. 2016).

In vitro shoot-tip cultures have been most commonly employed for chemical mutagenesis especially with ethyl methanesulphonate (EMS). Omar et al. (1989) observed that fresh weight and number of newly initiated adventitious buds from shoot tips of banana clones SH-3362 (AA) and GN-60A. For banana, EMS dose suggested for shoot tips was 12.41–37.23 mM with 1–3 h duration. Bhagwat and Duncan (1998a, b) compared the effects of sodium azide ($NaN_3$), diethyl sulphate (DES) and EMS on in vitro shoot tips of banana (Musa spp., AAA Group cv. Highgate) and found that the mutagens differed in their mutation induction efficiency. While $NaN_3$ showed the highest effectiveness (7.8%) resulting in 63.3% explant survival and 58.9% shoot regeneration, DES yielded 65.5% survival with 38.2% shoot regeneration and EMS


**Fig. 1.** Isolation of dwarf mutant in banana. **a** Giant cavendish control plant **b** 10 Gy gamma ray derived dwarf mutant

gave 5.8% effectiveness, 80% explant survival and 31.6% shoot regeneration. Bidabadi et al. (2012) treated shoot-tip explants with EMS and found 10-14% increase in phenotypic variation with time and dose of treatment. Pestanana et al. (2011) applied gamma rays and evaluated genetic variability for short height in putative banana 'Pacovan' (AAB genome, subgroup Prata type) mutants. Saraswathi et al. (2016) have successfully isolated three putative mutants resistant to Fusarium wilt. In vitro proliferating buds of Rasthali (Silk, AAB) were treated with EMS, NaN3 and DES (2, 0.02 and 0.15%), and then the mutated explants were screened in vitro against Fusarium wilt using fusaric acid and culture filtrate, followed by pot screening which led to isolation of resistant mutants.

Banana embryogenic cell suspension cultures are considered a good system for in vitro mutagenesis (Kulkarni et al. 2004; Jankowicz-Cieslak et al. 2012). Embryogenic cell suspension is of single-cell origin, and hence, the number of regenerated of chimeric plants can be reduced allowing rapid generation of homo-histonts or non-chimeric plants. Thus, ECS-based mutagenesis system should be the right choice for accelerating induction, selection and recovery of mutations (Suprasanna et al. 2008, 2012). Mutagenesis of shoot tips of banana cv. Nanicao (*Musa* cv. AAA group, Cavendish subgroup) with gamma rays followed by in vitro selection with 10 mM aluminium chloride resulted in the isolation of aluminium-tolerant banana plants (Matsumoto and Yamaguchi 1990). Roux and Toloza (2002) gamma-irradiated shoot cultures and selected plants for resistance to black Sigatoka. Banana cv. Grande Naine were irradiated at 35 Gy gamma rays, and after four in vitro passages were subjected to early mass screening by using juglone which is a toxic metabolite of *Mycosphaerella fijiensis*. The authors isolated 15 putative mutants showing black Sigatoka tolerance. Chen et al. (2013) developed a microcross section (MCS) culture system for EMS mutagenesis of Brazilian banana and selected banana plants for Fusarium wilt resistance. Five wilt-resistant lines were isolated suggesting that MCS system has good potential for use in mutagenesis and in vitro propagation. There are several successful examples on the isolation of mutants of different genomic groups for various agronomic traits (Table 10.1). Despite significant outcome on mutagenesis and mutant isolation, there have been few commercial releases of mutants as varieties (Table 10.2, Mutiara and Novaria by United Plantation Bhd in Malaysia) which may be due to long generation time of the crop, availability of a good in vitro system, handling of chimeras, non-stable genetic variability and continued efforts for field evaluation of mutant clones. Sustained research is warranted to realize the potential of mutation breeding to isolate, select, evaluate and develop commercially useful mutants in banana.

## 10.3 Somaclonal Variation

Edible banana and plantains are propagated vegetatively, and most of the genotypes are sterile (Heslop-Harrison and Schwarzacher 2007). Banana crop is severely affected by abiotic and biotic factors which limit its full production capability.

**Table 10.1** Studies on mutation induction using in vitro cultured shoot tips in treatment with chemical or physical mutagens in banana (modified after Jain et al. (2011))

| Cultivar/genomic group | Mutagen dose | Modification of trait(s) | Mutant/clones developed | Reference |
|---|---|---|---|---|
| *Chemical mutagens* | | | | |
| SH-3362 (AA); GN-60A (mutant of Grande Naine-AAA) | EMS 24.69 mM | Number of newly initiated adventitious buds decreased with increased EMS concentrations | – | Omar et al. (1989) |
| Highgate AAA Group | – Sodium azide ($NaN_3$) 2.3 mM<br>– Diethyl sulphate (DES)-20 mM<br>– EMS 200 mM | Tolerance to *Fusarium oxysporum* f.sp. *cubense* | Tolerant clones for field screening | Bhagwat and Duncan (1998a) |
| Brazilian banana | EMS-microcross section culture system | Tolerance to *Fusarium oxysporum* f.sp. *cubense* | Five wilt-resistant lines | Chen et al. (2013) |
| 'Berangan Intan', 'Berangan' (AAA group) and 'Rasthali' (AAB group) | EMS 200 mM EMS | Morphological variations | Morphological variations | Bidabadi et al. (2012) |
| Rasthali (Silk, AAB) | 2% EMS, 0.02% $NaN_3$ and 0.15% DES | Fungal disease resistance | Fusarium wilt-resistant mutants | Saraswathi et al. (2016) |
| *Physical mutagens* | | | | |
| Grande Naine AAA | Gamma rays 25 Gy | Bunch size and cylindrical shape | Klue Hom Thong KU1 | Anonymous (1990) |
| Nanicao AAA | Gamma rays 2kR | Aluminium tolerance | Tolerant lines | Matsumoto and Yamaguchi (1990) |
| Diploid and tetraploid clones | Gamma rays 25 Gy | Diploid clones were more sensitive than tetraploids | Plants with morphological and physiological traits | Novak et al. (1990) |
| Grande Naine AAA | Gamma rays | Earliness | Early flowering putative mutant designated 'GN-60A' | Roux (2004) |

(continued)

**Table 10.1** (continued)

| Cultivar/genomic group | Mutagen dose | Modification of trait(s) | Mutant/clones developed | Reference |
|---|---|---|---|---|
| Highgate (AAA) | Gamma rays 8-20 Gy | Tolerance to *Fusarium oxysporum* f. sp. *cubense* | Tolerant clones for field screening | Bhagwat and Duncan (1998b) |
| Basrai AAA | Gamma rays | Morphological traits | Clones for different morphological traits | Kulkarni et al. (1997) |
| Grande Naine AAA | Gamma rays 35 Gy | Resistance to black Sigatoka | 15 putative mutants | Roux and Toloza (2002) |
| Dwarf Parfitt, an extra dwarf Cavendish banana | Gamma rays 20 Gy | Improved agronomic characteristics (taller plant size, increased yield and no choking) | Improved lines with productivity and resistance | Smith et al. (2006) |
| Williams' and 'Cavendish Enano' | Carbon ion beam 0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 Gy | Fungal disease resistance | Resistant plants to black Sigatoka | Reyes-Borja et al. (2007) |
| Basari (AAA), Chakkarakela (AAB) and Rasthali (AAB) | Gamma rays 30 Gy (recurrent dose) | Morphological traits | Thick shiny dark green leaves, ovate leaves and a dwarf with a rosette of leaves | Mishra et al. (2007) |
| AAA group Giant Cavendish | Gamma rays (10, 30 Gy) | Morphological traits | Short height, early maturity | Ganapathi et al. (2008, 2016) |
| Dwarf Cavendish (AAA) | Gamma rays 8–20 Gy | Morphological traits | 22 clones for different morphological traits | Miri et al. (2009) |
| Banana 'Pacovan' (AAB genome, subgroup Prata | Gamma rays 20 Gy | Morphological traits | Short height | Pestanana et al. (2011) |

This has resulted in a concerted effort to genetically improve this crop. Conventional breeding is difficult in banana owing to its low female fertility and complex ploidy (Bakry et al. 2009). Biotechnological approaches complement the conventional breeding techniques for genetic enhancement of this crop. This includes in vitro multiplication, somatic embryogenesis, somaclonal variation, mutation breeding and genetic manipulation. Somaclonal variation has been quite effective approach in generating useful variations in vegetatively propagated plants such as banana, papaya, strawberry and watermelon (Jain et al. 2013; Krishna et al. 2016). Somaclonal vari-

**Table 10.2** Examples of desirable variants/putative mutants identified for release or further confirmation trials (Jain et al. 2011)

| Country | Parent/selection | Traits | Technique | Place of induction |
|---|---|---|---|---|
| Cuba | SH3436 (AAAB)/SH3436 | Reduced height | Gamma rays | Cuba |
| | Parecido al Rey (AAA)/Parecido al Rey 6.44 | Reduced height | Gamma rays | IAEA |
| Malaysia | Pisang Rasthali (AAB)/Mutiara | Tolerance to Foc race 4 | Somaclones | United Plantation Bhd., Malaysia |
| | Grande Naine GN-GoA (AAA)/Novaria | Tolerance to Foc race 4 | Somaclones | |
| | Pisang Berangan | Early flowering and reduced height | Somaclones | |
| | Pisang Berangan | Tolerance to Foc race 4 | Gamma rays | IAEA |
| | Pisang Mas | Tetraploid | Colchicine | |
| Philippines | Lakatan (AAA) | Reduced height and earliness | Gamma 40 Gy | IAEA |
| | Latundan (AAB) | Large fruit size and reduced height | 3 Gy fast neutrons | |
| Sri Lanka | Embul (AAB)/Embul | Earliness and reduced height | Gamma rays | Sri Lanka |

ations are either genetic or epigenetic that are observed in plants regenerated from tissue culture (Morrison et al. 1988; Evans 1989; Karp 1995; Brar and Jain 1998; Kaeppler et al. 2000). These variations occur due to changes in chromosome number, insertions, deletions, mutations, translocations, transposon activity and changes in the DNA methylation profile which can eventually lead to increased or decreased vigour and changes in qualitative or quantitative characteristics (Bairu et al. 2011; Krishna et al. 2016). Nevertheless, the epigenetic changes are lost after transfer from the culture conditions or multiplication after a few generations (Smulders and de Klerk 2011). Thus, the success of somaclonal variations in crop improvement is completely dependent on the genetic stability of the clones.

Somaclonal variations are quite common in banana with an average observed frequency of 6% at the phenotypic level (Vuylsteke et al. 1991). While some of the banana genotypes show higher rate of somaclonal variation, other genotypes have low rate of variation (Côte et al. 1995; Smith 1988; Vuylsteke et al. 1991) However, Hwang and Tang (2000) reported a somaclonal variation frequency as high as 69% in various banana and plantain cultivars. Various factors during tissue culture phase affect the incidence of somaclonal variations that includes genotype, type of explant, number of subculture cycles and plant growth regulators in the medium (Bairu et al.

2006). Several somaclones of banana have been isolated with varying visible morphological characters such as size and colour of pseudostem, reduced height, varying leaf size and variegation, bunch length and bunch mass (Israeli et al. 1991). Dwarf off-types are quite common among the tissue culture-derived Cavendish cultivars (Reuveni et al. 1986; Walduck et al. 1988; Hwang and Ko 1987; Damasco et al. 1997). In banana, dwarf mutants showed reduced height, thicker pseudostem and shorter but wider leaves (e.g. 'Cachaco Enano', 'Prataana' and 'Figue Rose naine') (Daniells et al. 2001). Drew and Smith (1990) observed that dwarf off-types in the tissue culture-regenerated banana cultivar New Guinea Cavendish (*Musa* sp., AAA Group, Cavendish subgroup) were stable up to five generations. However, in case of micropropagated 'False Horn' plantains, morphological variation in inflorescence type in the form of reversion to a typical 'French' plantain bunch type was variable from 0.4 to 100% of the total variability (Vuylsteke et al. 1988) which explains epigenetic variation. It is clearly observed that somaclonal variations are more profound in plants originated from differentiated tissues such as stem, leaves, roots or flowers and less when meristematic tissues were used as explants (De Klerk 1990; Skirvin et al. 1994).

Detection of somaclonal variation can be done using different morphological, cytological markers or molecular markers such as randomly amplified polymorphic DNA (Damasco 1997; Grajal-Martin et al. 1998; Giménez et al. 2001; Sheidai et al. 2008), amplified fragment length polymorphism (Engelborghs et al. 1998) and inter-simple sequence repeats (Lakshmanan et al. 2007). Somaclonal variation has dual considerations: it is not advantageous for micropropagation and germplasm preservation since maintenance of genetic uniformity is a must, whereas it can be advantageous for creating additional genetic variability for use in genetic improvement of banana. A somaclone CIEN BTA-03 was obtained from cultivar Williams (susceptible to black Sigatoka) and was micropropagated via apical shoot culture for five multiplication cycles. The CIEN BTA-03 clones demonstrated resistance to black Sigatoka and showed infection indexes similar to those of the resistant cultivar Yangambi Km5 (Trujillo and Garcia 1996; Giménez et al. 2008). Similarly, many somaclonal variants were developed having moderate to complete resistance to Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Taiwan Banana Research Institute (TBRI) developed somaclonal variants of the Giant Cavendish clone Pei Chiao and Tai Chiao No. 2 by using tissue culture. The mass-produced plantlets (called GCTCV) were tested for resistance against Fusarium wilt disease strain tropical race 4 (TR4) by planting them in TR4-infested fields and monitored for symptoms of Fusarium wilt. Although around 12 GCTCV clones were found to be more resistant to TR4 than Pei Chiao, these clones had poor agronomic traits making them unsuitable for the export trade. Since TBRI continuously supplied clones to farmers for plantation, a farmer from south Taiwan identified a high-yielding cultivar which was resistant to Foc TR4. This clone was labelled as GCTCV 218 and commercialized in Taiwan in the year 2001 under the name formosana (Tang 2001; Hwang and Ko 2004). In yet another study, somatic embryo-derived banana plants cv. Rasthali were maintained under tissue culture conditions for more than ten years (Ghag et al. 2014d). These plants were tested for resistance to Foc race 1, and five of

them did not show any Fusarium wilt symptoms after repeated bioassay. Molecular basis of resistance was investigated using cDNA-RAPD, and the differential bands were identified and characterized by quantitative RT-PCR. It was observed that a gene involved in jasmonic acid pathway, namely lipoxygenase, was downregulated in these resistant plants as compared to the susceptible controls indicating that lipoxygenase negatively regulates resistance response (Ghag et al. 2014d). These plants are currently being multiplied for field studies.

Banana and plantains are susceptible to low temperatures, nutrition deficiency, drought and poor light. However, some banana somaclones were also developed and tested for tolerance to low temperature and better yield under poor light conditions (Damasco et al. 1997). Several agronomically important characters go unnoticed when tissue culture plants are raised for a different purpose where somaclonal variation is undesirable for clonal propagation. However, somaclonal variation is a novel source for use in crop improvement in vegetatively propagated plants such as banana. Extensive studies are needed for the selection of desirable characters and field evaluation to prove their stability over multiple generations with superior performance.

## 10.4 Novel Genetic Variation Through Transgenics

Development of efficient cell and tissue culture protocols and successful transformation methods has enabled genetic engineering a possible reality in banana. Genetic transformation has been optimized in different banana and plantain cultivars using different methods. First transformation of banana (*Musa* spp., cv. 'Bluggoe', ABB group) was performed by electroporating protoplast cultures obtained from embryogenic cell suspension cultures (Sagi et al. 1994). First transgenic banana plants were generated from embryogenic cell suspension cultures using the particle gun method (Côte et al. 1995; Sagi et al. 1995; Escalant et al. 1995). But the most popular method of transformation of different banana tissues has been the *Agrobacterium*-mediated transformation (May et al. 1995; Ganapathi et al. 2001; Khanna et al. 2004; Tripathi et al. 2005).

Genetic modification has always been a lucrative strategy for banana improvement because most edible elite cultivars of bananas have poor male and female fertility hindering conventional breeding, thereby preventing introgression of desired characters in these cultivars. On the other hand, transgenic technology provides a wide gene pool for choosing the desired genes which can be selectively transferred to the banana cultivar. Several laboratories across the world, namely BARC (India), QUT (Australia), IITA (Kenya), NARO (Uganda) and KU Leuven (Belgium) have developed transgenic banana plants with improved character/s and demonstrated their potential under greenhouse or even in field trials. Plant-based vaccines are another application of plant genetic engineering for human health (Gujjula et al. 2004, 2007). In a first report, Kumar et al. (2005) reported expression of hepatitis B surface antigen in transgenic banana fruits. Transgenic banana plants have been developed targeting resistance to pest and pathogens, abiotic stress tolerance and delayed fruit ripening

(Ghag and Ganapathi 2017). The gene/s used to impart these traits into transgenic banana plants were either from banana plants or identified from other sources (Tripathi et al. 2010; Paul et al. 2011; Ghag et al. 2012, 2014a; Shekhawat et al. 2011, 2013; Sreedharan et al. 2013a, b). Large expanse of information has now become available to us due to the genome sequencing of important banana cultivars such as *Musa acuminata* (D'Hont et al. 2012), *Musa balbisiana* (Davey et al. 2013) and *Musa itinerans* (Wu et al. 2016). Concurrently, transcriptome and proteome data in response to resistance–susceptibility (Li et al. 2012; Bai et al. 2013), abiotic stress sensitivity tolerance (Yang et al. 2015; Muthusamy et al. 2016) and fruit ripening (Asif et al. 2014) has also been generated. All these sequencing programmes will have to provide better understanding and identification of gene/s present in banana genome which can be used to impart characteristic traits in transgenic banana plants. Table 10.3 lists some of the developments on genetically modified banana for improved traits.

Recent technologies such as RNA interference (Ghag et al. 2014b; Elitzur et al. 2016) and metabolic engineering (Paul et al. 2017) have been successfully performed in banana using different genes targeting important traits, whereas the latest genome-editing technology CRISPR-Cas is currently under research. Host-induced gene silencing, an approach which employs RNA interference technology, was used to develop resistance to one of the most devastating diseases of banana, the Fusarium wilt. DNA fragments of some vital genes (Velvet protein gene and FTF1 gene) from Fusarium wilt pathogen were identified and transferred to banana plants using *Agrobacterium*-mediated genetic transformation. These fragments were oriented in the construct such a way that resulted in generation of double stranded small RNA molecules which inhibited the pathogen growth and development in susceptible banana cultivars imparting high-level resistance (Ghag et al. 2014b). Moreover, RNA interference was also used to confer resistance in transgenic banana plants to Banana bunchy top disease (Shekhawat et al. 2013). But these studies were restricted to the greenhouse. Few studies did advance to the field trials including resistance to bacterial wilt and nematodes, biofortification and delayed ripening.

Banana Xanthomonas wilt is yet another problem constraining banana production, and resistance to this disease was demonstrated in transgenic banana plants by expressing two genes, namely the hypersensitive response-assisting protein (*Hrap*) and the plant ferredoxin-like protein gene (*Pflp*). These transgenic banana lines showed complete resistance under glasshouse as well as field conditions (Tripathi et al. 2014a, b). Transgenic plantains expressing cysteine proteinase inhibitor and an anti-root invasion, non-lethal synthetic peptide demonstrated 99% resistant to banana nematodes *Radopholus similis* and *Helicotylenchus multicinctus,* and the transgenic plants had equivalent agronomic performance as compared to the non-transgenic counterparts (Tripathi et al. 2015).

Biofortification programmes have always been forerunners in developing regions as alternatives to adequate food supply and supplementation. Very recently, provitamin A biofortified bananas were developed by metabolic engineering using different constructs having phytoene synthase gene (Paul et al. 2017). Checking postharvest losses by controlling ripening and extending shelf-life in banana is one of the approaches to improve food security. In this regard, transgenic Cavendish bananas

**Table 10.3** List of transgenic banana plants generated with improved characters

| Target gene | Transgenic plant/cultivar | Improved character | References |
|---|---|---|---|
| *Abiotic stress tolerance* | | | |
| *Musa-DHN-1* | *Musa* spp. *Rasthali* | Drought, salt tolerance | Shekhawat et al. (2011) |
| *MusaSAP1* | *Musa* spp. *Rasthali* | Drought, salt, oxidative stress tolerance | Sreedharan et al. (2012) |
| *MusaPIP1;2* | *Musa* spp. *Rasthali* | Cold, salt, drought tolerance | Shreedharan et al. (2013a, b) |
| *MusaWRKY71* | *Musa* spp. *Rasthali* | Salt, oxidative stress tolerance | Shekhawat et al. (2013) |
| *MusabZIP53* | *Musa* spp. *Rasthali* | Cold, drought, salt tolerance | Shekhawat et al. (2014) |
| *MaPIP1;1* | *Arabidopsis* | Salt, drought tolerance | Xu et al. (2014) |
| *AhSIPR10* | *M. acuminata* cv. *Matti* | Salt, drought tolerance | Rustagi et al. (2015) |
| *MusaPIP2;6* | *Musa* spp. *Rasthali* | Salt tolerance | Sreedharan et al. (2015) |
| *MpMYBS3* | *Musa* spp. cv. *Brazil* | Cold tolerance | Dou et al. (2016) |
| *MusaNAC042* | *Musa* spp. *Rasthali* | Salt, drought tolerance | Tak et al. (2017) |
| *MusaNAC68* | *Musa* spp. *Rasthali* | Salt, drought tolerance | Negi et al. (2016) |
| *Biotic stress tolerance* | | | |
| Magainin | *Musa* spp.cv. *Rasthali* | Resistance to Foc race 1 | Chakrabarti et al. (2003) |
| *HL* | *M.* spp. cv. *Taijiao* | Resistance to Foc race 4 | Pei et al. (2005) |
| *Endo β-1,3-glucanase gene* | *M.* spp.cv. *Rasthali* | Resistance to Foc race 1 | Maziah et al. (2007) |
| *pflp* | *M. acuminata* cv. *Pei Chiao*<br>*M. acuminate* cv. *Gros Michel* | Resistance to Foc race 4 | Yip et al. (2011) |
| *Bcl-xL, Ced-9 and Bcl-2 3'UTR* | *M.* spp. cv. *Lady Finger* | Resistance to Foc race 1 | Paul et al. (2011) |
| *tlp* | *M.* spp. cv. Nangka | Resistance to Foc race 4 | Mahdavi et al. (2012) |
| *PhDef1, PhDef2* | *M.* spp. cv. *Rasthali* | Resistance to Foc race 1 | Ghag et al. (2012) |

(continued)

**Table 10.3** (continued)

| Target gene | Transgenic plant/cultivar | Improved character | References |
|---|---|---|---|
| *chit42* | *M.* spp. cv. *Furenzhi* | Resistance to Foc race 4 | Hu et al. (2013) |
| *Ace-AMP1* | *M.* spp. cv. *Rasthali* | Resistance to Foc race 1 | Mohandas et al. (2013) |
| *VEL* and *FTF1* | *M.* spp. cv. *Rasthali* | Resistance to Foc race 1 | Ghag et al. (2014a) |
| *Sm-AMP-D1* | *M.* spp. cv. *Rasthali* | Resistance to Foc race 1 | Ghag et al. (2014b) |
| *MusaDAD1*, *MusaBAG1* and *MusaBI1* | *M.* spp. cv. *Rasthali* | Resistance to Foc race 1 | Ghag et al. (2014c) |
| *ThEn-42* and *StSy* | *M.* spp. cv. *Grand Nain* | Resistance to *Mycosphaerella fijiensis* | Vishnevetsky et al. (2011) |
| *rcc2, rcg3* | *M.* spp. cv. *Gros Michel* | Resistance to *Mycosphaerella fijiensis* | Kovács et al. (2013) |
| *Pflp*, *hrap* | *M.* spp. cv. *Sukali Ndizi* and *M.* spp. cv. *Nakinyika* | Resistance to *X. campestris* pv. *musacearum* | Tripathi et al. (2014a) |
| *Xa21* | *M.* spp. cv. *Gonja manjaya* | Resistance to *X. campestris* pv. *musacearum* | Tripathi et al. (2014b) |
| *Rep* | *M.* spp. cv. *Dwarf Brazilian* | Resistance to *BBTV* | Cheah et al. (2009) |
| *BBTV-G- cp* | *M.* spp. cv. Williams | Resistance to *BBTV* | Ismail et al. (2011) |
| *Rep, ProRep* | *M.* spp. cv. *Rasthali* | Resistance to *BBTV* | Shekhawat et al. (2013) |
| *Cystatin* | *M.* spp. | Resistance to *Radopholus similis* | Atkinson et al. (2003) |
| *Cystatin*, synthetic peptide | *M.* spp. cv. *Gonja manjaya* | *R. similis, Helicotylenchus multicinctus* and *Meloidogyne spp* | Roderick et al. (2012) |
| *Biofortification* | | | |
| *Soyferritin* | *M.* spp. cv. *Rasthali* | Enhanced iron content in leaves of transgenic plants | Sunil Kumar et al. (2011) |
| *MtPsy2a/ZmPsy1* | *M. acuminata* cv. *Dwarf Cavendish* | Enhanced levels of pro-vitamin A | Paul et al. (2017) |

[a] *Foc*—Fusarium oxysporum f. sp. cubense, *BBTV*—banana bunchy top virus

were generated by repressing the MADS box genes *MaMADS*1/*MaMADS*2 using RNAi technology. These plants were evaluated under field conditions and displayed delayed ripening and extended shelf-life phenotypes that include delayed colour development and softening (Elitzur et al. 2016). Although most of these studies have undergone field trials and have proved their excellence, none of them are yet commercialized. This is because of the scare spawned among the general public about transgenics or genetically modified foods. Some of the transgenic bananas have already been field tested for its improved traits, nutritional and agronomic performance (Ghag and Ganapathi 2017). The recent technologies such as CRISPR-Cas9 are useful in editing genome for value-added traits without introgression of foreign genes.

## 10.5 Banana Genomics

The draft genome sequence of banana has been made available through the sequencing efforts of the doubled haploid genome of *Musa acuminate*—DH Pahang (D'Hont et al. 2012). The sequence represents 90% of the genome and has 36542 protein-coding genes and 37 microRNA families, and half of the sequence composed of transposable elements. Davey et al. (2013) reported the draft genome for *Musa balbisiana* Pisang Klutuk Wulung (PKW). This group has used Illumina HiSeq 2000 II technology and generated 281 million, 100 bp paired-end Illumina reads, and the reads were assembled using the already available reference A genome. The group has identified 36638 protein-coding genes and 3276 transposable elements. The available sequence data can be effectively used for genome editing/manipulation for abiotic and biotic stress tolerance as well as for fruit quality improvement in banana. The data revealed that banana genome had most genes located in the distal part of its chromosomes, and akin to other plant genomes, banana also has major chunk (50%) of transposable elements. D'Hont et al. (2012) also observed highest number (3155) of transcription factors of which 759 (MYB and AP2/ERF type) are specific to banana. Ghag et al. (2015) conducted small RNA expression profiling in two commercially important banana cultivars and identified several cultivar specific miRNAs along with putative target transcripts. In a further study, Harikrishna et al. (2016) analysed stress-related miRNA, and their predicted targets in the banana A and B genomes and their results suggest that siRNA expression patterns change in response to salt stress. The post-genomics research is poised to provide greater insights and boost to Musa genetic engineering and improvement through the identification, cloning and functional characterization of useful genes for key agronomic traits (Dash and Rai 2016; Ghag and Ganapathi 2017).

## 10.6 Conclusions

Banana is an important food crop after the major cereals like rice, wheat and maize. Currently, several biotechnological tools are being applied for improving the *Musa*

germplasm. In the early 2000, the Global Musa Genomics Consortium was established with the prime focus on efficient use of Musa biodiversity for sustainability through the integrated approaches of genetic and genomic resources, precision breeding and genetic transformation. The crop is also susceptible to biotic and abiotic stress factors, and hence, there is a greater need to aim for improvement to better adapt to the changing climatic environment. For vegetatively propagated plant like banana, induced mutations offer as a potential for generating novel variability. In vitro mutagenesis has been successfully adopted followed by production of mutant population, mutation screening and phenotype characterization. The establishment of appropriate transgenic methods has contributed banana improvement. Several useful gene constructs, promoters and other regulatory elements have been made available for achieving stress-resistant/tolerant plants. Current and near-future improvement strategies for developing cold, drought-tolerant bananas can augment efforts for growing under the climate change challenges and thus can contribute substantially to food security.

## References

Anonymous (1990) List of new mutant cultivars; *Musa* sp. (banana). Mutat Breeding Newsl. 35:32–41

Asif MH, Lakhwani D, Pathak S, Gupta P, Bag SK, Nath P, Trivedi PK (2014) Transcriptome analysis of ripe and unripe fruit tissue of banana identifies major metabolic networks involved in fruit ripening process. BMC Plant Biol 14:316

Atkinson HJ, Grimwood S, Johnston K, Green J (2004) Prototype demonstration of transgenic resistance to the nematode *Radopholus similis* conferred on banana by a cystatin. Transgenic Res 13:135–142

Bai TT, Xie WB, Zhou PP, Wu ZL, Xiao WC, Zhou L, Sun J, Ruan XL Li HP (2013) Transcriptome and expression profile analysis of highly resistant and susceptible banana roots challenged with *Fusarium oxysporum* f. sp. *cubense* tropical race 4. PLoS ONE 8:e73945

Bairu MW, Aremu AO, Van Staden J (2011) Somaclonal variation in plants: causes and detection methods. Plant Growth Regul 63:147–173

Bairu MW, Fennell CW, van Staden J (2006) The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Musa* AAA cv. 'Zelig'). Sci Hortic 108:347–351

Bakry F, Carreel F, Jenny C, Horry JP (2009) Genetic improvement of banana. In: Breeding plantation tree crops: tropical species, pp 3–50

Bhagwat B, Duncan EJ (1998a) Mutation breeding of banana cv. Highgate (*Musa acuminata*, AAA Group) for tolerance to *Fusarium oxysporum* f. sp. *cubense* using chemical mutagens. Sci Hortic 73:11–22

Bhagwat B, Duncan EJ (1998b) Mutation breeding of Highgate (*Musa acuminata*, AAA) for tolerance to *Fusarium oxysporum* f. sp. *cubense* using gamma irradiation. Euphytica 101:143–150

Bidabadi SS, Meon S, Wahab Z, Subramaniam S, Mahmood M (2012) Induced mutations for enhancing variability of banana (*Musa* spp.) shoot tip cultures using ethyl methanesulphonate (EMS). Aust J Crop Sci 6:567–575

Brar, D, Jain SM (1998). Somaclonal variation: mechanism and applications in crop improvement. In: Somaclonal variation and induced mutations in crop improvement. Springer, Netherlands, pp 15–37

Chakrabarti A, Ganapathi TR, Mukherjee PK, Bapat VA (2003) MSI-99, a magainin analogue, imparts enhanced disease resistance in transgenic tobacco and banana. Planta 216:587–596

Cheah K, Chen Y, Xie WS, Gaskill D, Khalil S, Sether D, Melzer M, Wang M, Manshardt R, Borth W, Perez E (2009) Transgenic banana plants resistant to banana bunchy top virus infection. In: V International symposium on banana: ISHS-ProMusa symposium on global perspectives on asian challenges, 897, pp 449–457

Cheesman EE (1949) Classification of the bananas: critical notes on species: *M. textilis*. Kew Bulletin, pp 267–272

Chen YF, Chen W, Huang X, Hu X, Zhao JT, Gong Q, Li XJ, Huang XL (2013) Fusarium wilt-resistant lines of Brazil banana (*Musa* spp., AAA) obtained by EMS-induced mutation in a micro-cross-section cultural system. Plant Pathol 62:112–119

Côte FX, Grapin A, Legavre T, Rabot B, Monmarson S, Frigout O, Teisson C (1995) Genetic transformation of banana and plantain by particle bombardment: I. Transformation of cell suspensions

Creste S, Neto AT, Vencovsky R, de Oliveira Silva S, Figueira A (2004) Genetic diversity of *Musa* diploid and triploid accessions from the Brazilian banana breeding program estimated by microsatellite markers. Genet Resour Crop Evol 51:723–733

Cronauer SS, Krikorian AD (1984) Multiplication of *Musa* from excised stem tips. Ann Bot 53(3):321–328

Damasco OP (1997) Molecular methods for early identification of banana (*Musa* spp., AAA) dwarf somaclonal variants. Philippine Technol J 22:29–39

Damasco OP, Smith MK, Adkins SW, Hetherington SE, Godwin ID (1997) Identification and characterisation of dwarf off-types from micropropagated Cavendish bananas. In: II international symposium on banana: i international symposium on banana in the subtropics, 490, pp. 79–84

Damasco OP, Smith MK, Godwin ID, Adkins SW, Smillie RM, Hetherington SE (1997b) Micropropagated dwarf off-type Cavendish bananas (*Musa* spp., AAA) show improved tolerance to suboptimal temperatures. Aust J Agric Res 48:377–384

Daniells JC, Karamura JD, Tomekpe K (2001) Musalogue: a catalogue of musa germplasm. Diversity in the genus musa, international network for the improvement of banana and plantain, Montpellier, France. ISBN 2-910810-42-9

Dash PK, Rai R (2016) Translating the "Banana Genome" to delineate stress resistance, dwarfing, parthenocarpy and mechanisms of fruit ripening. Front. Plant Sci. 7:1543

Davey MW, Gudimella R, Harikrishna JA, Sin LW, Khalid N, Keulemans J (2013) A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter-and intra-specific *Musa* hybrids. BMC Genom 141:683

De Guzman EV, Ubalde EM, Del Rosario AG (1976) Banana and coconut in-vitro cultures for induced mutations studies (No. IAEA–194)

Dou TX, Hu CH, Sun XX, Shao XH, Wu JH, Ding LJ et al (2016) MpMYBS3 as a crucial transcription factor of cold signaling confers the cold tolerance of banana. Plant Cell Tissue Organ Cult 125:93–106

De Klerk GJ (1990) How to measure somaclonal variation. Acta Botanica Neerlandica 39:129–144

D'hont A, Denoeud F, Aury JM, Baurens FC, Carreel F, Garsmeur O, Noel B, Bocs S, Droc G, Rouard M, Da Silva C, et al (2012) The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. Nature 488:213

Drew RA, Smith MK (1990) Field evaluation of tissue-cultured bananas in south-eastern Queensland. Aust J Exp Agric 30:569–574

Elitzur T, Yakir E, Quansah L, Zhangjun F, Vrebalov JT, Khayat E, Giovannoni JJ, Friedman H (2016) Banana MaMADS transcription factors are necessary for fruit ripening and molecular tools to promote shelf-life and food security. Plant Physiology 01866

El-Tarras AE, Taha FA, Abd-el-Wahab S (1995) Somaclonal variation in micropropagated banana at biochemical and chromosomal levels. Bulletin of Faculty of Agriculture Cairo Univ (Egypt), Egypt

Engelborghs I, Swennen R, Van Campenhout S (1998) The potential of AFLP to detect genetic differences and somaclonal variants in *Musa* spp. Infomusa 7:3–6

Escalant JV, Teisson C, Cote FX (1994) Amplified somatic embryogenesis from male flowers of triploid banana and plantain cultivar (*Musa* spp). Vitro Cell Dev Biol. 30:181–186

Escalant JV, Legavre T, Ortiz JL, Teisson C (1995) Genetic transformation of banana and plantain by particle bombardment: II. Transformation of somatic embryos
Evans DA (1989) Somaclonal variation-genetic basis and breeding applications. Trends Genet 5:46–50
FAO (2015) Food and Agriculture Organization of the United Nations http://faostat.fao.org
FAO Agriculture Data (2002) http://www.fao.org/ag
Frison E, Sharrock, SL (1998) The economic, social and nutritional importance of banana in the world. In: Picq C, Foure E, Frison EA (eds) Bananas and Food Security. Proceedings on international symposium on Douala, Cameroon, 10–14 Nov 1998, INIBAP, Montpellier, France, pp 21–35
Ganapathi TR, Suprasanna P, Bapat VA, Kulkarni VM, Rao PS (1999) Somatic embryogenesis and plant regeneration from male flower buds in banana. Curr Sci 76:1228–1231
Ganapathi TR, Higgs NS, Balint-Kurti PJ, Arntzen CJ, May GD, Van Eck JM (2001) *Agrobacterium*-mediated transformation of embryogenic cell suspensions of the banana cultivar Rasthali (AAB). Plant Cell Rep 20:157–162
Ganapathi TR, Sidha M, Suprasanna P, Ujjappa KM, Bapat VA, D'Souza SF (2008) Field performance and RAPD analysis of gamma-irradiated variants of banana cultivar 'Giant Cavendish' (AAA). Int J Fruit Sci 8:147–159
Ganapathi TR, Ujjappa KM, Badigannavar A (2016) Characterization of Gamma Ray Induced Clones in 'Giant Cavendish' Banana (AAA) for morphological and yield contributing traits. Int J Fruit Sci 16:310–322
Ghag SB, Ganapathi TR (2017) Genetically modified bananas: to mitigate food security concerns. Sci Hortic 214:91–98
Ghag SB, Shekhawat UK, Ganapathi TR (2014a) Characterization of Fusarium wilt resistant somaclonal variants of banana cv Rasthali by cDNA-RAPD. Mol Biol Rep 41:7929–7935
Ghag SB, Shekhawat UKS, Ganapathi TR (2012) *Petunia* floral defensins with unique prodomains as novel candidates for development of Fusarium wilt resistance in transgenic banana plants. PLoS ONE 7:e39557
Ghag SB, Shekhawat UKS, Ganapathi TR (2014b) Host-induced post-transcriptional hairpin RNA-mediated gene silencing of vital fungal genes confers efficient resistance against Fusarium wilt in banana. Plant Biotechnol J 12:541–553
Ghag SB, Shekhawat UKS, Ganapathi TR (2014c) Transgenic banana plants expressing a *Stellaria media* defensin gene (*Sm-AMP-D1*) demonstrate improved resistance to *Fusarium oxysporum*. Plant Cell Tissue Organ Cult 119:247–255
Ghag SB, Shekhawat UKS, Ganapathi TR (2014d) Native cell-death genes as candidates for developing wilt resistance in transgenic banana plants. AoB Plants 6:plu037
Ghag SB, Shekhawat UKS, Ganapathi TR (2015) Small RNA profiling of two important cultivars of banana and overexpression of miRNA156 in transgenic banana plants. PLoS ONE 10(5):e0127179. https://doi.org/10.1371/journal.pone.0127179
Gill, LS (1988) Taxonomy of flowering plants. In: Ibadan (ed) Africana-Fep Publishers Ltd, pp 106–109
Giménez C, de García E, Haddad O (2008) Genetic and resistance stability to Black Sigatoka disease during micropopagation of *Musa* CIEN BTA-03 somaclonal variant. Phyton (Buenos Aires), p. 65
Giménez C, De Garcia E, De Enrech NX Blanca I (2001) Somaclonal variation in banana: cytogenetic and molecular characterization of the somaclonal variant CIEN BTA-03. In vitro Cellular Dev Biol-Plant 37:217–222
Gujjula SKB, Ganapathi TR, Bapat VA (2004) Edible vaccines: current status and future prospects. Physiol Mol Biol Plants 10(1):37–47
Gujjula SKB, Ganapathi TR, Bapat VA (2007) Production of hepatitis B surface antigen in recombinant plant systems: an update. Biotechnol Prog 23(3):532–539
Grajal-Martin M, Siverio-Grillo G, Marrero-Dominguez A (1998) The use of randomly amplified polymorphic DNA (RAPD) for the study of genetic diversity and somaclonal variation in Musa. In:

II International Symposium on Banana: I International Symposium on Banana in the Subtropics, 490, pp 445–454
Harikrishna JA, Lee WS, Gudimella R, Khalid N, Davey MW (2016) The banana salt stress transcriptome: large and small RNA expression in stressed banana. Acta Hortic 1114:125–132
Helliot B, Panis B, Poumay Y, Swennen R, Lepoivre P, Frison, E (2002) Cryopreservation for the elimination of cucumber mosaic and banana streak viruses from banana (*Musa* spp.). Plant Cell Rep 20(12):1117–1122
Hwang SC, KO WH (1987) Somaclonal variation of bananas and screening for resistance to Fusarium wilt. In: ACIAR Proceedings Series, Australian Centre for International Agricultural Research 21, pp 151–156
Hwang SC, Ko WH, Chao CP (1994) GCTCV-215-1: a promising cavendish clone resistant to race 4 of fusarium oxysporum f. sp. cubense. Plant Prot Bull (Taiwan) 36:281–291
Heslop-Harrison JS, Schwarzacher T (2007) Domestication, genomics and the future for banana. Ann Bot 100:1073–1084
Hirano T, Kazama Y, Ishii K, Ohbu S, Shirakawa Y, Abe T (2015) Comprehensive identification of mutations induced by heavy-ion beam irradiation in Arabidopsis thaliana. Plant J 82:93–104
Hu CH, Wei YR, Huang YH, Yi GJ (2013) An efficient protocol for the production of chit42 transgenic Furenzhi banana (*Musa* spp. AA group) resistant to *Fusarium oxysporum*. In vitro Cellular Dev Biol-Plant, 49:584–592
Hu W, Ding Z, Tie W, Yan Y, Liu Y, Wu C, Liu J, Wang J, Peng M, Xu B, Jin Z (2017) Comparative physiological and transcriptomic analyses provide integrated insight into osmotic, cold, and salt stress tolerance mechanisms in banana. Sci Rep 7:43007
Hwang SC, Ko WH (1987) Somaclonal variation of bananas and screening for resistance to Fusarium wilt. Banana Plantain Breeding Strat pp 151–156
Hwang SC, Ko WH (2004) Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan. Plant Dis 88:580–588
Hwang S, Tang CY (2000) Improved resistance to Fusarium wilt through somaclonal variation in cavendish bananas. In: Proc. Conf. on Challenges for banana production and utilization in 21st Century, September 24–25, 1996 Trichy, India. Association for improvement in production and utilization of Banana (AIPUB) pp 195–208
INIBAP (2006) Global conservation strategy for musa (Banana and Plantain) INIBAP is a network of bioversity international, a center of a consultative document prepared by INIBAP with the collaboration of numerous partners in the musa research-and-development community
Ismail RM, El-Domyati FM, Wagih EE, Sadik AS, Abdelsalam AZE (2011) Construction of banana bunchy top nanovirus-DNA-3 encoding the coat protein gene and its introducing into banana plants cv. Williams. J Genetic Eng Biotechnol 9:35–41
Israeli Y, Reuveni O, Lahav E (1991) Qualitative aspects of somaclonal variations in banana propagated by in vitro techniques. Sci Hortic 48:71–88
Jain SM, Swennen, R (2004) Banana improvement: cellular, molecular biology and induced mutations. Sci. Publishers, Inc., Enfield, NH, USA
Jain SM, Roux N, Penna S, Till B (2011) Mutations and cultivar development of Banana. In: Pillay M, Tenkouano A (eds) Banana breeding: progress and challenges book chapter. CRC Press, Taylor & Francis, pp 201–215
Jain SM, Brar DS, Ahloowalia BS (eds) (2013) Somaclonal variation and induced mutations in crop improvement, vol 32. Springer Science & Business Media
Jankowicz-Cieslak J, Huynh OA, Brozynska M, Nakitandwe J, Till BJ (2012) Induction, rapid fixation and retention of mutations in vegetatively propagated banana. Plant Biotechnol J 10:1056–1066
Jarret RL, Gawel N, Whittemore A, Sharrock S (1992) RFLP-based phylogeny of *Musa* species in Papua New Guinea. Theor Appl Genet 84:579–584
Kaeppler SM, Kaeppler HF, Rhee Y (2000) Epigenetic aspects of somaclonal variation in plants. Plant Mol Biol 43:179–188

Karmarkar VM, Kulkarni VM, Suprasanna P, Bapat VA, Rao PS (2001) Radio-sensitivity of in vivo and in vitro cultures of banana cv. Basrai (AAA). Fruits 56:67–74

Karp A (1995) Somaclonal variation as a tool for crop improvement. Euphytica 85:295–302

Khanna H, Becker D, Kleidon J, Dale J (2004) Centrifugation assisted *Agrobacterium tumefaciens*-mediated transformation (CAAT) of embryogenic cell suspensions of banana (*Musa* spp. Cavendish AAA and Lady finger AAB). Mol Breeding 14:239–252

Kotari P, Swarupa V, Ravishankar K (2016) Genomics of Biotic Stress Tolerance in Banana. In: Mohandas, S, Ravishankar K (eds.) Banana: genomics and transgenic approaches for genetic improvement, pp 61–75

Kovács G, Sági L, Jacon G, Arinaitwe G, Busogoro JP, Thiry E, Strosse H, Swennen R, Remy S (2013) Expression of a rice chitinase gene in transgenic banana ('Gros Michel', AAA genome group) confers resistance to black leaf streak disease. Transgenic Res 22:117–130

Krishna H, Alizadeh M, Singh D, Singh U, Chauhan N, Eftekhari M, Sadh RK (2016) Somaclonal variations and their applications in horticultural crops improvement. 3 Biotech 6:54

Kulkarni VM, Suprasanna P, Bapat VA (2006) Plant regeneration through multiple shoot formation and somatic embryogenesis in a commercially important and endangered Indian banana cv. Rajeli Curr Sci 90(6):842–846

Kulkarni VM, Ganapathi TR, Bapat VA, Rao PS (2004) Establishment of cell suspension cultures in banana cv. Grand Naine and evaluation of its sensitivity to gamma-irradiation. Curr Sci 86:902–904

Kulkarni VM, Ganapathi TR Suprasanna P, Bapat VA (2007) In vitro mutagenesis in banana (*Musa* spp.) using gamma irradiation. In: Jain Sm, Haggman H (eds) Protocols for micropropagation of woody trees and fruits. Springer Publishers, The Netherlands, pp. 543–559

Kulkarni VM, Ganapathi TR, Suprasanna P, Bapat VA, Rao PS (1997) Effect of gamma irradiation on in vitro multiple shoot cultures of banana (*Musa* species). J. Nuclear Agri Biol 26:232–240

Kumar GB, Ganapathi TR, Bapat VA (2007) Production of hepatitis B surface antigen in recombinant plant systems: an update. Biotechnol Prog 23:532–539

Kumar GBS, Srinivas L, Ganapathi TR (2011) Iron fortification of banana by the expression of soybean ferritin. Biol Trace Elem Res 142:232–241

Kumar GS, Ganapathi TR, Bapat VA (2004) Edible vaccines: current status and future prospects. Physiol Molecular Biol Plants 10:37–47

Kumar GS, Ganapathi TR, Revathi CJ, Srinivas L, Bapat VA (2005) Expression of hepatitis B surface antigen in transgenic banana plants. Planta 222:484–493

Lakshmanan V, Venkataramareddy SR, Neelwarne B (2007) Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR marker. Electron J Biotech 10:1–8

Langridge WR (2000) Edible Vaccines. Scientific American

Li CY, Deng GM, Yang J, Viljoen A, Jin Y, Kuang RB, Zuo, CW, Lv ZC, Yang QS, Sheng O, Wei YR (2012) Transcriptome profiling of resistant and susceptible Cavendish banana roots following inoculation with *Fusarium oxysporum* f. sp. *cubense* tropical race 4. BMC Genomics 13:374

Mahdavi F, Sariah M, Maziah M (2012) Expression of rice thaumatin-like protein gene in transgenic banana plants enhances resistance to Fusarium wilt. Appl Biochem Biotechnol 166:1008–1019

Matsumoto K, Yamaguchi H (1990) Selection of aluminium-tolerant variants from irradiated protocorm-like bodies in banana. Tropical Agriculture 67:229–232

May GD, Afza R, Mason HS, Wiecko A, Novak FJ, Arntzen CJ (1995) Generation of transgenic banana (*Musa acuminata*) plants via *Agrobacterium*-mediated transformation. Nat Biotechnol 13:486–492

Mishra PJ, Ganapathi TR, Suprasanna P, Bapat VA (2007) Effect of single and recurrent irradiation on in vitro shoot cultures of banana. Int J Fruit Sci 7(1):47–57

Maziah M, Sariah M, Sreeramanan S (2007) Transgenic banana *Rastali* (AAB) with β-1, 3-glucanase gene for tolerance to Fusarium wilt race 1 disease via *Agrobacterium*-mediated transformation system. Plant Pathol J 6:271–282

Menendez T (1973) A note on the effect of ethyl-methanesulphonate on *Musa acuminata* seeds, induced mutations in vegetatively propagated plants, 85-90. IAEA, Vienna, Austria

Miri SM, Mousavi A, Naghavi MR, Mirzaii M, Talaei AR, Naserian Khiabani B (2009) Analysis of induced mutants of salinity Resistant banana (Musa acuminata cv. Dwarf Cavendish) using morphological and molecular markers. Iran J Biotechnol 7(2):86–92

Menon R (2016) Banana breeding. In: Mohandas S, Ravishankar K (eds) Banana: genomics and transgenic approaches for genetic improvement. Springer, Singapore

Mohandas S, Sowmya HD, Saxena AK, Meenakshi S, Rani RT, Mahmood R (2013) Transgenic banana cv. *Rasthali* (AAB, Silk gp) harboring *Ace-AMP1* gene imparts enhanced resistance to *Fusarium oxysporum* f. sp. *cubense* race 1. Sci Hortic 164:392–399

Morita R, Kusaba M, Iida S, Yamaguchi H, Nishio T, Nishimura M (2009) Molecular characterization of mutations induced by gamma irradiation in rice. Genes Genetic Syst 84:361–370

Morpurgo R, Brunner H, Grasso G, Van Duren M, Roux N, Afza R (1997) Enigma of banana breeding: a challenge for biotechnology. Agro-Food Ind Hi-Tech 16–21

Morrison RA, Whitaker RJ, Evans DA (1988) Somaclonal variation: its genetic basis and prospects for crop improvement. In: Opportunies for phytochemistry and plant biotechnology. Plenum Publishing Corporation, New York, pp 1–18

Muthusamy M, Uma S, Backiyarani S, Saraswathi MS, Chandrasekar A (2016) Transcriptomic changes of drought-tolerant and sensitive banana cultivars exposed to drought stress. Front Plant Sci **7**

Navarro C, Escobedo RM, Mayo A (1997) In vitro plant regeneration from embryogenic cultures of a diploid and a triploid, Cavendish banana. Plant Cell Tissue Organ Cult 51:17–25

Negi S, Tak H, Ganapathi TR (2016) Expression analysis of *MusaNAC68* transcription factor and its functional analysis by overexpression in transgenic banana plants. Plant Cell Tissue Organ Cult (PCTOC) 125:59–70

Novak FJ, Afza R, Duren MV, Omar MS (1990) Mutation induction by gamma irradiation of in vitro cultured shoot-tips of banana and plantain (*Musa* cvs). Trop Agri. 67:21–28

Orjeda G (2000) Evaluating bananas: a global partnership. Results of IMTP Phase II, INIBAP, Montpellier, France

Omar MS, Novak FJ, Brunner H (1989) In vitro action of ethyl-methanesulphonate on banana shoot tips. Sci Hortic 40(1989):283–295

Ortiz R (1995) Musa Genetics. In: Gowen S (ed) Bananas and plantains, Chapman and Hall, London pp 84–109

Ortiz R, Ferris RSB, Vuylsteke DR (1995) Banana and plantain breeding. In: Gowen S (ed) Bananas and plantains 110-146. Springer, Netherlands

Osuji JO, Harrison G, Crouch J, Heslop-Harrison JS (1997) Identification of the genomic constitution of *Musa* L. lines (bananas, plantains and hybrids) using molecular cytogenetics. Ann Bot 80:787–793

Oselebe HO, Tenkouano A, Pillay M (2006) Ploidy variation of *Musa hybrids* from crosses. Afr J Biotechnol, Nairobi 5:1048–1053

Othman RY, Shadia AR, Chai M (2002) Ploidy analysis and DNA content of mutant banana "Pisang Berangan" using flow cytometry. Biotropia-the Southeast Asian J Tropical Biol 19

Padam BS, Tin HS, Chye FY, Abdullah MI (2014) Banana by-products: an under-utilized renewable food biomass with great potential. J Food Sci Technol 51:3527–3545

Patel P, Yadav K, Ganapathi TR (2016) Biofortification for alleviating iron deficiency anemia. In: Banana: genomics and transgenic approaches for genetic improvement. Springer Science Business Media Singapore, pp 301–338

Paul JY, Becker DK, Dickman MB, Harding RM, Khanna HK, Dale JL (2011) Apoptosis-related genes confer resistance to Fusarium wilt in transgenic 'Lady Finger' bananas. Plant Biotechnol J 9:1141–1148

Paul JY, Khanna H, Kleidon J, Hoang P, Geijskes J, Daniells J, Zaplin E, Rosenberg Y, James A, Mlalazi B, Deo P (2017) Golden bananas in the field: elevated fruit pro-vitamin A from the expression of a single banana transgene. Plant Biotechnol J 15:520–532

Pei XW, Chen SK, Wen RM, Ye S, Huang JQ, Zhang YQ, Wang BS, Wang ZX, Jia SR (2005) Creation of transgenic bananas expressing human lysozyme gene for Panama wilt resistance. J Integr Plant Biol 47:971–977

Pestanana RKN, Amorim EP, Ferreira CF, Amorim VB, de O, Oliveira LS, Ledo CA, da S, Silva S, de O.E (2011) Agronomic and molecular characterization of gamma ray induced banana (*Musa* sp.) mutants using a multivariate statistical algorithm. Euphytica 178:151–158

Pillay, M, Tripathi L (2007) Banana. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 4, Fruits and Nuts. Springer-Verlag, Berlin Heidelberg, pp 281–301

Ravi I, Uma S, Vaganan MM, Mustaffa MM (2013) Phenotyping bananas for drought resistance. Front Physiol 4:9

Ravi I, Vaganan MM (2016) Abiotic stress tolerance in nanana. In: Rao N, Shivashankara K, Laxman R (eds) Abiotic stress physiology of horticultural crops. Springer, New Delhi

Reuveni O, Israeli Y, Degani H, Eshdat Y (1986) Genetic variability in banana plants multiplied via in vitro techniques. IBPGR Final Report, 36

Reyes-Borja WO, Sotomayor I, Garzon I et al (2007) Alteration of resistance to black sigatoka (Mycosphaerella fijiensis Morelet) in banana by in vitro irradiationusing carbon ion-beam. Plant Biotechnol J 24:349–353

Robinson JC (1996) Bananas and plantains. CAB International, Wallingford, UK

Roderick H, Tripathi L, Babirye A, Wang D, Tripathi J, Urwin PE, Atkinson HJ (2012) Generation of transgenic plantain (*Musa* spp.) with resistance to plant pathogenic nematodes. Mol Plant Pathol 13:842–851

Roux NS (2004) Mutation induction in *Musa*—Review. In: Jain SM, Swennen R (eds) Banana improvement: cellular, molecular biology, and induced mutations. Science Publishers, New Hampshire, USA, pp 23–32

Roux NS and Toloza A (2002) Update in resistance to black sigatoka through induced mutations. Proceedings of the 15th international meeting ACROBAT Cartagena de Indias, Colombia, October 27–November 2, 2002

Roux N, Dolezel J, Swennen R, Zapata-Arias FJ (2001) Effectiveness of three micropropagation techniques to dissociate cytochimeras in *Musa* spp. Plant Cell Tissue Organ Cult 66:189–197

Rustagi A, Jain S, Kumar D, Shekhar S, Jain M, Bhat V, Sarin NB (2015) High efficiency transformation of banana [*Musa acuminata* L. cv. *Matti* (AA)] for enhanced tolerance to salt and drought stress through overexpression of a peanut salinity-induced pathogenesis-related class 10 protein. Mol Biotechnol 57:27–35

Ryuto H, Fukunishi N, Hayashi Y, Ichida H, Abe T, Kase M, Yano Y (2008) Heavy-ion beam irradiation facility for biological samples in RIKEN. Plant Biotechnol 25:119–122

Sági L, Panis B, Remy S, Schoofs H, De Smet K, Swennen R, Cammue BP (1995) Genetic transformation of banana and plantain (*Musa* spp.) via particle bombardment. Nat Biotechnol 13:481–485

Sagi L, Remy S, Panis B, Swennen R, Volckaert G (1994) Transient gene expression in electroporated banana (*Musa* spp., cv. 'Bluggoe', ABB group) protoplasts isolated from regenerable embryogenetic cell suspensions. Plant Cell Rep 13:262–266

Saraswathi MS, Kannan G, Uma S, Thangavelu R, Backiyarani S (2016) Improvement of banana cv. *Rasthali* (Silk, AAB) against *Fusarium oxysporum* f. sp. *cubense* (VCG 0124/5) through induced mutagenesis: Determination of LD50 specific to mutagen, explants, toxins and in vitro and in vivo screening for Fusarium wilt resistance

Sheidai M, Aminpoor H, Noormohammadi Z, Farahani F (2008) RAPD analysis of somaclonal variation in banana (*Musa acuminate* L.) cultivar Valery. Acta Biol Szeged 52:307–311

Shekhawat UK, Ganapathi TR, Hadapad AB (2012) Transgenic banana plants expressing small interfering RNAs targeted against viral replication initiation gene display high-level resistance to banana bunchy top virus infection. J Gen Virol 93:1804–1813

Shekhawat UKS, Ganapathi TR (2013) *MusaWRKY71* overexpression in banana plants leads to altered abiotic and biotic stress responses. PLoS ONE 8:e75506

Shekhawat UKS, Ganapathi TR (2014) Transgenic banana plants overexpressing *MusabZIP53* display severe growth retardation with enhanced sucrose and polyphenol oxidase activity. Plant Cell Tissue Organ Cult 116:387–402

Shekhawat UKS, Srinivas L, Ganapathi TR (2011) *MusaDHN-1*, a novel multiple stress-inducible SK3-type dehydrin gene, contributes affirmatively to drought-and salt-stress tolerance in banana. Planta 234:915–932

Sidha M, Shinde BN, Bapat VA, Suprasanna P (2011) Plant regeneration through somatic embryogenesis from male flower buds and assessment of clonal fidelity in banana cv. Lal Kela (AAA). J Fruit Ornamental Plant Res 19(2):15–30

Singh B, Singh JP, Kaur A, Singh N (2016) Bioactive compounds in banana and their associated health benefits-A review. Food Chem 206:1–11

Simmonds NW (1966) Bananas. London:Longmans p 512

Simmonds NW, Shepherd K (1955) Taxonomy and origins of cultivated bananas. J Linn Soc London Bot 55:302–312

Skirvin RM, McPheeters KD, Norton M (1994) Sources and frequency of somaclonal variation. HortScience 29:1232–1237

Smith MK (1988) A review of factors influencing the genetic stability of micropropagated bananas. Fruits 43:219–223

Smith MK, Hamill SD, Langdon PW, Giles JE, Doogan VJ, Pegg KG (2006) Towards the development of a Cavendish banana resistant to race 4 of fusarium wilt: gamma irradiation of micropropagated Dwarf Parfitt (*Musa* spp., AAA group, Cavendish subgroup). Aust J Exp Agric 46:107–113

Smulders MJM, de Klerk GJ (2011) Epigenetics in plant tissue culture. Plant Growth Regul 63:137–146

Stotzky G, Cox EA, Goos RD, Wornick RC, Badger AM (1964) Some effects of gamma irradiation on seeds and rhizomes of *Musa*. Am. J. Botany 724–729

Stover RH, Simmonds NW (1987) Bananas. Tropical Agriculture Series, Longman, Harlow, Essex, pp 407–422

Stover RH, Simmonds NW (1987) Classification of banana cultivars. In: Stover RH and Simmonds NW (ed.) Bananas, 3rd edn. Wiley, New York, 97–103

Sreedharan S, Shekhawat UK, Ganapathi TR (2013) Transgenic banana plants overexpressing a native plasma membrane aquaporin MusaPIP1; 2 display high tolerance levels to different abiotic stresses. Plant Biotechnol J 11:942–952

Sreedharan S, Shekhawat UKS, Ganapathi TR (2015) Constitutive and stress-inducible overexpression of a native aquaporin gene (*MusaPIP2; 6*) in transgenic banana plants signals its pivotal role in salt tolerance. Plant Mol Biol 88:41–52

Sreedharan S, Shekhawat UKS, Ganapathi TR (2012) *MusaSAP1*, a A20/AN1 zinc finger gene from banana functions as a positive regulator in different stress responses. Plant Mol Biol 80:503–517

Strosse H, Schoofs H, Panis B, Andre E, Reyniers K, Swennen R (2006) Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.). Plant Sci 170:104–112

Suprasanna P, Pania B, Sági L, Swennen R. (2001) Establishment of embryogenic cell suspension cultures from Indian banana cultivars. In: 3rd and Final Research Coordination Meeting of the FAO/IAEA on Cellular biology of banana. Leuven, Belgium pp 11–12

Suprasanna P, Meenakshi S, Bapat VA (2008) Integrated approaches of mutagenesis and in vitro selection for crop improvement. In: Kumar A, Shekhawat NS (eds) Plant tissue culture, molecular markers and their role in crop productivity. IK International Publishers, New Delhi, India, pp 73–92

Suprasanna P, Jain SM, Ochatt SJ, Kulkarni VM, Predieri S (2012) Applications of *in vitro* techniques in mutation breeding of vegetatively propagated crops. In: Shu QY, Forster BP, Nakagawa H (eds) Plant mutation breeding and biotechnology. CABI Publishing, Wallingford, pp 371–385

Tak H, Negi S, Ganapathi TR (2017) Banana NAC transcription factor *MusaNAC042* is positively associated with drought and salinity tolerance. Protoplasma 254:803–816

Tang CY (2001) Somaclonal variation: a tool for the improvement of Cavendish banana cultivars. In: II international symposium on biotechnology of tropical and subtropical species, 692, pp 61–66

Till BJ, Cooper J, Tai TH, Colowit P, Greene EA, Henikoff S, Comai L (2007) Discovery of chemically induced mutations in rice by TILLING. BMC Plant Biol 7:19

Till BJ, Jankowicz-Cieslak J, Sági L, Huynh OA, Utsushi H, Swennen R, Terauchi R, Mba C (2010) Discovery of nucleotide polymorphisms in the *Musa* gene pool by Ecotilling. Theor Appl Genet 121:1381–1389

Tripathi JN, Lorenzen J, Bahar O, Ronald P, Tripathi L (2014a) Transgenic expression of the rice Xa21 pattern-recognition receptor in banana (*Musa* sp.) confers resistance to Xanthomonas campestris pv. musacearum. Plant Biotechnol J 12:663–673

Tripathi L, Babirye A, Roderick H, Tripathi JN, Changa C, Urwin PE, Tushemereirwe WK, Coyne D, Atkinson HJ (2015) Field resistance of transgenic plantain to nematodes has potential for future African food security. Scientific Reports, 5

Tripathi L, Mwaka H, Tripathi JN, Tushemereirwe WK (2010) Expression of sweet pepper *Hrap* gene in banana enhances resistance to *Xanthomonas campestris* pv. musacearum. Molecular Plant Pathology 11:721–731

Tripathi L, Tripathi JN, Hughes JDA (2005) *Agrobacterium*-mediated transformation of plantain (*Musa* spp.) cultivar Agbagba. African J Biotechnol 4

Tripathi L, Tripathi JN, Kiggundu A, Korie S, Shotkoski F, Tushemereirwe WK (2014b) Field trial of Xanthomonas wilt disease-resistant bananas in East Africa. Nat Biotechnol 32:868–870

Trujillo I, Garcia E (1996) Strategies for obtaining somaclonal variants resistant to yellow Sigatoka (*Mycosphaerella musicola*). Infomusa 5:12–13

Van Harten AM (1998) Mutation breeding: theory and practical applications. Cambridge University Press, Cambridge

Vishnevetsky J, White TL Jr, Palmateer AJ, Flaishman M, Cohen Y, Elad Y, Velcheva M, Hanania U, Sahar N, Dgani O, Perl A (2011) Improved tolerance toward fungal diseases in transgenic Cavendish banana (*Musa* spp. AAA group) cv. Grand Nain Transgenic Res 20:61–72

Vuylsteke D, Swennen R, De Langhe E (1991) Somaclonal variation in plantains (*Musa* spp., AAB group) derived from shoot-tip culture. Fruits (France)

Vuylsteke D, Swennen R, Wilson GF, De Langhe E (1988) Phenotypic variation among in-vitro propagated plantain (*Musa* sp. cultivar 'AAB'). Sci Hortic 36:79–88

Vuylsteke D, Swennen R, Ortiz R (1993) Registration of 14 improved tropical Musa plantain hybrids with black sigatoka resistance. HortScience 28:957–959

Walduck G, Daniells J, Gall E (1988) Results of survey of off-types in tissue cultured Cavendish bananas in North Queensland 1987. Bananatopics 8:11–12

Wang TL, Uauy C, Robson F, Till B (2012) TILLING in extremis. Plant Biotechnol J 10:761–772

Wu W, Yang YL, He WM, Rouard M, Li WM, Xu M, Roux N, Ge XJ (2016) Whole genome sequencing of a banana wild relative *Musa itinerans* provides insights into lineage-specific diversification of the *Musa* genus. Scientific Reports 6:31586

Xu Y, Hu W, Liu J, Zhang J, Jia C, Miao H, Xu B, Jin Z (2014) A banana aquaporin gene, *MaPIP1; 1*, is involved in tolerance to drought and salt stresses. BMC Plant Biol 14:59

Yang QS, Gao J, He WD, Dou TX, Ding LJ, Wu JH, Li CY, Peng XX, Zhang S, Yi GJ (2015) Comparative transcriptomics analysis reveals difference of key gene expression between banana and plantain in response to cold stress. BMC Genom 16:446

Yip MK, Lee SW, Su KC, Lin YH, Chen TY, Feng TY (2011) An easy and efficient protocol in the production of pflp transgenic banana against Fusarium wilt. Plant Biotechnol Rep 5:245–254