37 Somaclonal Variation in Clonal Crops: Containing the Bad, Exploring the Good

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Abstract

Somaclonal variation describes random cellular changes in plants regenerated through tissue culture. It occurs in certain crops that undergo micropropagation and has been recorded in different explant sources, from leaves and shoots to meristems and embryos. In banana (*Musa* spp.), a clonal crop conserved *in vitro*, somaclonal variation has been observed after prolonged periods in tissue culture, resulting from an increase in subcultures performed on a given clone. According to scientific literature, variants, or off-types, often show characteristics such as abnormal growth and flower or fruit defects in frequencies ranging from 1% to 32%. This variation poses a problem for gene bank managers, whose mandate is to maintain the genetic integrity of their collections for research and breeding. In the case of the Bioversity International *Musa* Germplasm Transit Centre (ITC), stress during the *in vitro* process is minimized by various techniques and plants are regenerated after 10 years, making it a long and costly process. Identifying somaclonal variation at an early stage would be an ideal solution; however, this requires suitable molecular markers. Recent studies revealed that techniques such as direct DNA sequencing and single nucleotide polymorphisms (SNPs) are able to detect the underlying factors of somaclonal variation and are becoming more accessible. On the other hand, somaclonal variation can be beneficial as it allows the natural development of new varieties and supplies genetic stocks used for future genetic studies. Harnessing the diversity of somaclones is easier, faster and cheaper compared with other methods of crop improvement, although it is also less predictable. So far, variants of crops such as apple, strawberry, potato and banana have been successfully adopted into global markets. In this chapter, we will discuss how to minimize the adverse effects of somaclonal variation while maximizing its benefits for greater crop diversity, with a particular focus on banana.

Keywords: clonal crops • somaclonal variation • crop improvement • mutation breeding • genetic stocks

1 Introduction

Vegetative or clonal propagation is an asexual reproduction in which successive mitosis of specialized vegetative propagules (as bulbs, corms, tubers, cuttings, buds and apomictic seeds) develops new plants and results in a clonal population. Clonal crops such as potatoes, yams, sweet potatoes, banana and cassava complement maize, rice, wheat, legumes, vegetables and livestock and provide income, nutrition and food security for around 300 million poor people in developing countries (Thiele *et al.*, 2017).

A clone is usually considered to be genetically uniform material derived from a single individual that is vegetatively propagated either

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in vivo or *in vitro*. *In vitro* rapid mass propagation (or micropropagation) has the main advantage of increasing the propagation rate. But tissue culture *in vitro* may also create undesired variation, also called somaclonal variation. The term 'somaclonal variation' was first introduced by Larkin and Scowcroft (1981) to describe the variation observed among plants regenerated after passing through tissue culture or cell culture. Somaclonal variations were first recorded in potato, sugarcane, rice and maize in the 1970s and 1980s (Karp, 1995). There appear to be two types of somaclonal variation: heritable and epigenetic (Skirvin *et al.*, 1994; Kaeppler *et al.*, 2000).

- Heritable variation is stable through the sexual cycle or repeated asexual propagation. Somaclonal variation can involve either single or multiple genes and can be due to alterations in DNA sequence, genes, chromosomes or entire sets of chromosomes.
- Epigenetic variation may be unstable even when asexually propagated. In this case, somaclonal variation involves mechanisms of gene silencing or gene activation that were not due to chromosome aberrations or sequence change.

According to reviews by Skirvin *et al.* (1994) and Krishna *et al.* (2016), the factors affecting somaclonal variation can be of different origins, such as:

- the tissue culture environment (e.g. temperature or light);
- the culture medium, including growth regulators such as auxins or kinetin;
- the explant source (tissue with preformed shoots are more stable than other types of explants such as adventitious buds with undifferentiated tissues);
- the plant genotype (within a species, the frequency of somaclonal variations usually occur at higher ploidy levels);
- the number of subcultures; and
- the time spent in tissue culture *in vitro* (not regenerated).

Crops that are propagated by tissue culture are more likely to display somaclonal variation. Among all the reviews written on somaclonal variation, Bairu *et al.* (2011) gave a useful list of 180 examples of plants with the sources of variation and reference. The list showed that most of the somaclonal variation occurs in species with breeding limitations (such as clonal crops) and are due to the preferred method of propagation via tissue culture. A selection of species from Bairu *et al.* (2011) is given in Table 37.1.

2 Challenges of Somaclonal Variation

2.1 In commercial production

Commercial micropropagation was initiated in the 1970s and 1980s, when the number of commercial laboratories undertaking these activities grew significantly. There were high expectations during that period and then in the 1990s many failures occurred due to the production of off-types (Reuveni *et al.*, 1993; Skirvin *et al.*, 1994). Even though many laboratories reduced their commercial tissue culture operations,

Table 37.1. A selection of five crops showing somaclonal variation (from Bairu *et al.*, 2011).

Species	Common name	Source of variation	Detection method	Reference
Allium sativum L.	Garlic	Genotype, colchicine treatment	Morphology, isozyme patterns	Chomatova *et al.* (1990)
Musa spp.	Banana	Number of subcultures	Morphology, RAPD, microsatellite markers	Ray *et al.* (2006)
Solanum tuberosum L.	Potato	Embryogenic culture	Morphology	Rietveld *et al.* (1991)
Fragaria L.	Strawberry	6-benzylaminopurine	Morphology, RAPD	Biswas *et al.* (2009)
Saccharum L.	Sugarcane	Callus culture	Morphology, susceptibility to red-rot disease	Singh *et al.* (2008)

such companies still exist (especially for ornamental plants) which follow best practices to avoid the high rate of somaclonal variation. Commercial companies multiply few diverse accessions at high rates.

2.2 In gene banks

For *in vitro* gene banks the situation is different from commercial laboratories since the goal is to conserve the highest possible diversity and maintain a limited number of plantlets per accession. To limit the number of subcultures and reduce as much as possible the manpower needed to maintain a high quantity of accessions, the cultures are maintained under slow growth conditions. For example, at Bioversity's International *Musa* Germplasm Transit Centre (ITC), accessions can be maintained for 1 year on average without subculturing if the conditions include reduced temperature and light, growth regulator in the medium and with minimal replication to maintain healthy germplasm. Gene banks multiply their accessions regularly and at rates depending on demand. Quality management systems are generally put in place to ensure that the distributed material is true to type. Somaclonal variants are not true-to-type accessions and therefore cannot be distributed by gene banks – this amounts to a loss of conserved/available genetic diversity. Gene banks must therefore detect somaclonal variants or 'off-types', eliminate them from the active collection and replace them from the original source if possible.

2.3 Detection and solutions

Commercial companies as well as gene banks have been investigating how to limit, as much as possible, the production of somaclonal variants. Based on the causes listed above, the following recommendations are made to limit the production of off-types/variants (Smith *et al.*, 1992).

- Select the ideal genotype or accession that shows relative stability as starting material or mother plant.
- Minimize stress through explant sources, regeneration techniques and culture environment.
- Limit subculture cycles and regenerate plants regularly. Restrict multiplication to approximately 1000 plants, which corresponds to around ten subcultures from initiation. If we consider a multiplication rate of two at each subculture, we should have, from each explant, $2^{10} = 1024$ plantlets after ten subcultures.

At the ITC, in order to perform the third recommendation above we estimated that we reach 1000 plants per meristem after ten subcultures, which corresponds to 10 years (as accessions are subcultured once per year on average).

During the early 2000s, the ITC initiated the so-called Field Verification exercise, which was put in place to field-verify all accessions that had been *in vitro* at the ITC for more than 10 years. The first step of the ongoing exercise is that three to five plantlets of accessions available for distribution and detected as virus-free are sent to the field, i.e. the field collection of the USDA Research Station in Puerto Rico (USDA-ARS-TARS). At least three plants per accession are grown in the field and 34 morphological descriptors are recorded (based on the minimum descriptors TAG, 2010) together with a set of ten standard photos agreed upon by a panel of taxonomists called the Taxonomic Advisory Group (TAG). All morphological data and photos, plus any comments from the USDA curator, are compiled in the *Musa* Germplasm Information System (MGIS) database (Ruas *et al.*, 2017). The data is then shared with the TAG panel, where each expert gives their opinion on the true-to-type nature of each ITC accession to determine its genetic integrity.

In parallel, the ITC collection (i.e. 1566 accessions) is being genotyped, using flow cytometry to determine the ploidy and using 19 SSR markers to record the genomic constitution (based on methods in Hippolyte *et al.*, 2012, and Christelová *et al.*, 2017). The entire process is depicted in Fig. 37.1.

The Field Verification process has enabled the detection of mislabelled or misclassified accessions, but not somaclonal variants. It was only after the publication of the whole *Musa* genome sequence (D'hont *et al.*, 2012) that we could map SNP markers to detect some cases of somaclonal variants. By aligning the genotyping by sequencing (GBS) data to the referenced sequenced genome, it was possible to visualize the distribution by chromosome of the ploidy

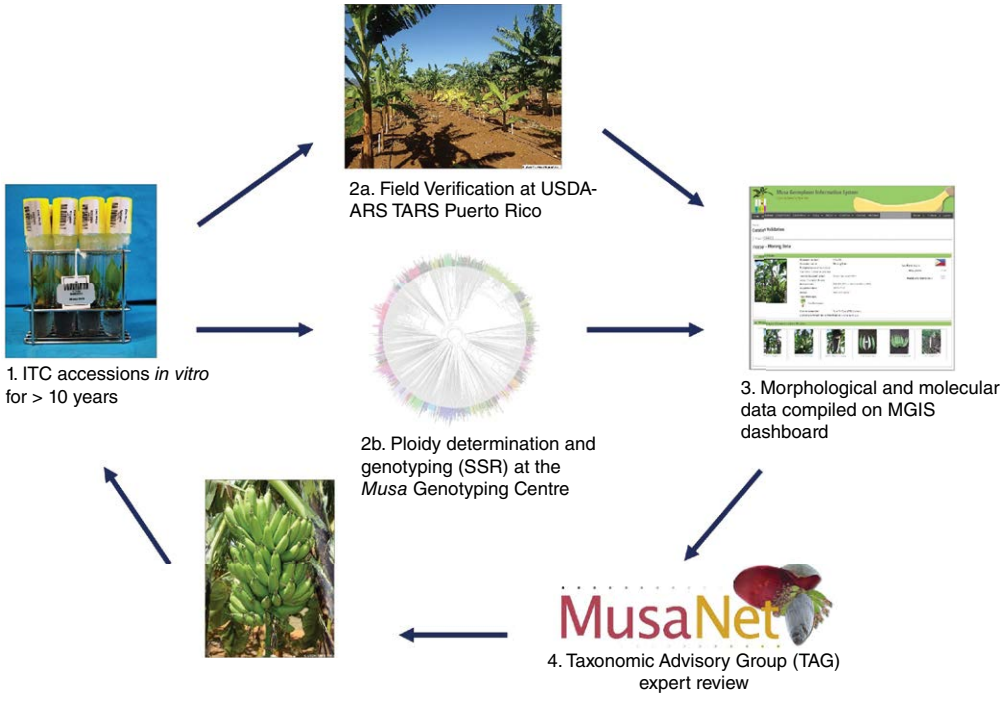

Fig. 37.1. The Field Verification process of ITC banana accessions.

ratio on biallelic SNP data, the ploidy ratio being the ratio for a given SNP, between the number of reads observed on the major allele divided by the total number of reads obtained for that given SNP (e.g. an SNP with A allele for 15 reads and C for 30 reads will have a ploidy ratio of 30/(30 + 15) = 0.666). For a typical diploid profile, all chromosomes are expected to exhibit a peak centred around 0.50 (Fig. 37.2a), and around 0.66 for a triploid (Fig. 37.2b); ratios of 0.75 are expected for AA×AB allotetraploids (Fig. 37.2c).

For most of the accessions, we have found that all the chromosomes matched with the overall expected ploidy. Nevertheless, we detected in some accessions that one or a few chromosomes had unexpected ploidy ratios. For example, when comparing the profile of the mother plant of plantain cultivar 'Ihitisim' (AAB) NGA-124 (originating from IITA field collection in Onne, Nigeria) maintained in the field but never propagated *in vitro*, with its daughter plant 'Ihitisim' (AAB) ITC0121 introduced *in vitro* at ITC in 1986, we detected that the chromosome profiles are not identical. For the accession NGA-124, the peaks of all 11 chromosomes are at a major allele frequency of approximately 0.66, as expected of a triploid (Fig. 37.3a). For the accession ITC0121, the peak of chromosome 3 is centred around 0.75, which corresponds to the expectation of a tetraploid profile for that specific chromosome (Fig. 37.3b).

These preliminary observations need more investigation on a larger number of accessions, but this method may only detect somaclonal variations that are due to aneuploidy or large chromosomal aberrations. Consequently, a routine somaclonal variation detection pipeline could be put in place in order to improve and accelerate the process of determining genetic fidelity of accessions maintained *in vitro* not only in gene banks, but also in commercial tissue culture laboratories.

3 Benefits of Somaclonal Variation

3.1 In crop improvement

Even though most somaclonal variants are of no value as they have deleterious traits, in some

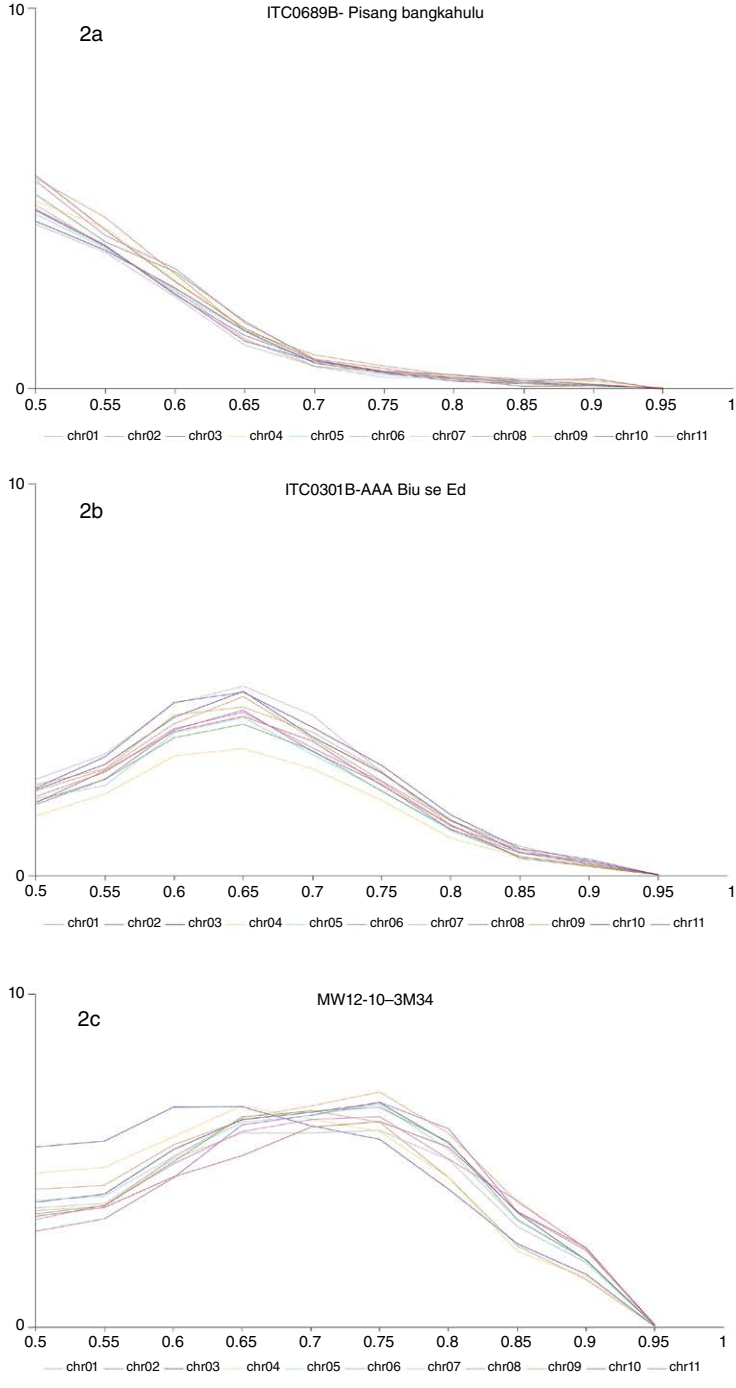

Fig. 37.2. Distribution for the 11 chromosomes of the ploidy ratio on biallelic SNP data. *X*-axis: consecutive classes of ploidy ratio calculated for a given SNP as the ratio of the number of reads with the major allele on the total number of reads obtained for that given SNP, ratio which varies between 0.5 and 1 according to this definition. *Y*-axis: frequency (in %) of SNPs observed along the chromosome for a given class of ploidy ratio.

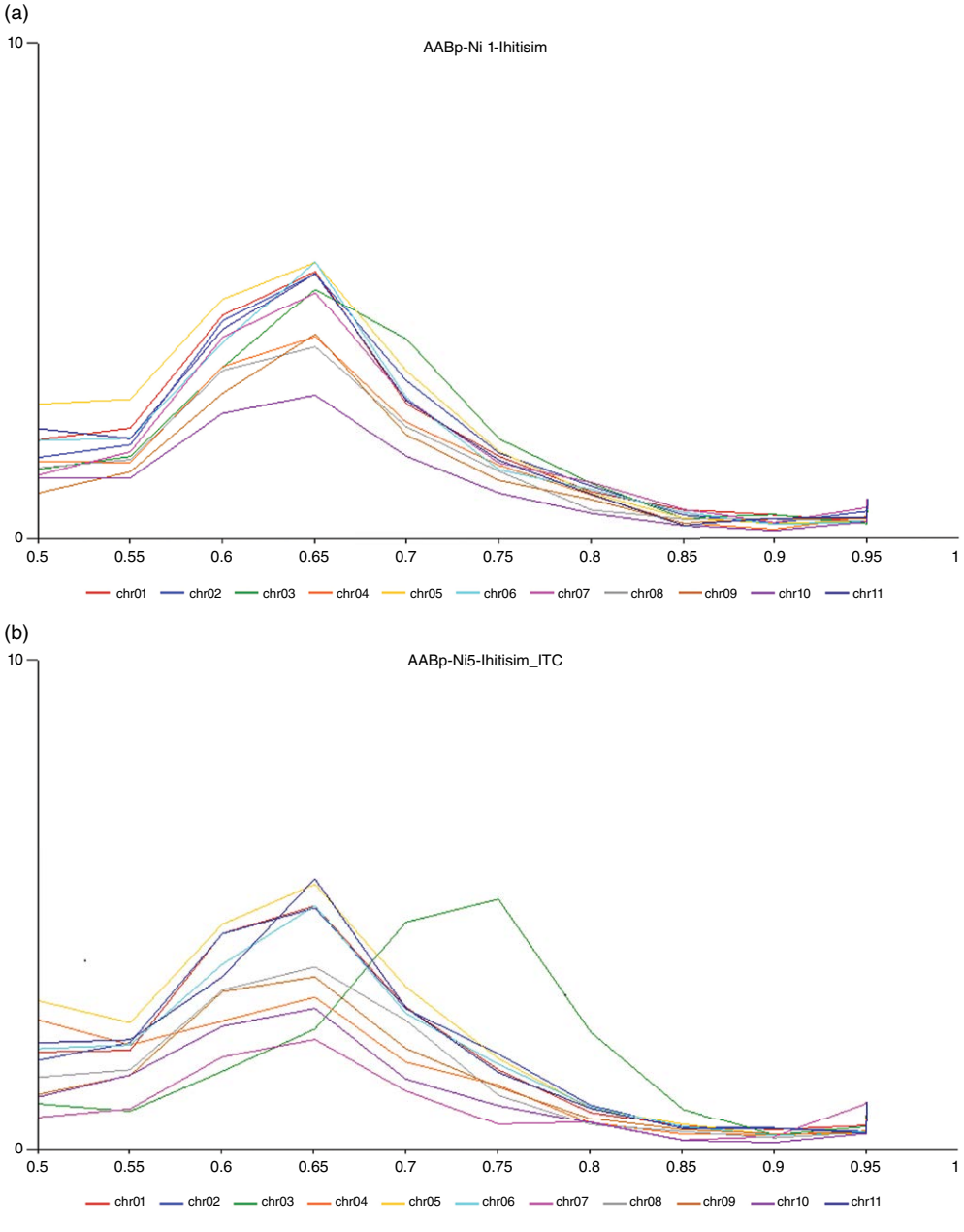

Fig. 37.3. **(a)** Ploidy distribution per chromosome of NGA-124 'Ihitisim' (AAB). All chromosomes are forming a peak at ratio 0.66 (2/3) which is expected from a triploid genotype. **(b)** Ploidy distribution per chromosome of ITC0121 'Ihitisim' (AAB). All chromosomes are forming a peak at ratio 0.66 except for chromosome 3 (in green) with a peak at ratio 0.75 corresponding to tetraploid profile.

cases they do produce improved traits and are even considered as an additional crop improvement method, very often compared with a mutation induction technique even though there are no physical or chemical mutagens involved. The advantage is that, when the variants are stable, they represent another source of genetic diversity that is important for crop improvement as

they provide new variability for breeders. Some examples are listed in Table 37.2.

3.2 The banana case study: 'Formosana' (GCTCV-218)

Researchers at the Taiwan Banana Research Institute (TBRI) have been investigating since the early 1980s how to obtain resistant varieties to Fusarium wilt disease caused by the fungus *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (FOC TR4). No fungicide can kill this pathogen, so selection of resistant varieties is the fastest way to prevent further damage. Conventional breeding for triploid cultivars such as the Cavendish, the subgroup representing 90% of the banana cultivated area in Taiwan, is difficult as they are sterile. Researchers from TBRI found that among the millions of plants distributed from tissue culture to farmers, about 3% displayed variation in size or colour of the pseudostem and leaves and in the shape of leaves and fruits (Hwang, 1986). In 1984, TBRI decided to initiate a project to screen Cavendish plantlets for FOC TR4 resistance that included four steps:

Step 1: The establishment of a FOC TR4-infested test plot
Step 2: Healthy plants are planted at high density with plants of a susceptible variety (Fig. 37.4a)
Step 3: Screening for somaclonal variants with resistance to FOC TR4 (Fig. 37.4b)
Step 4: Recurrent selection of an improved type (Fig. 37.4c).

'Formosana' was not the first somaclonal variant detected to be tolerant to FOC TR4; nevertheless, the general wilt incidence was 4.1%, which is significantly lower than the resistant 'Tai Chiao' with 9.5% and the 'Giant Cavendish' with 29.6% (Hwang and Ko, 2004). In addition, most of the agronomical characteristics of 'Formosana' (i.e. stronger pseudostem, thicker leaves, better hand formation and more uniform hand size and higher yield) make it a superior cultivar even if it takes one additional month to produce fruit as compared with its progenitor 'Pei Chiao'. Starting in 2000, 'Formosana' was distributed to farmers and was very well received for its resistance to FOC TR4 and its fruit quality. Commercial planting was possible after only 6 years of research. Although there are now millions of plants produced commercially, it is still not known what kind of variation occurred in the genome. With the sequenced information now available, new molecular markers and tools have been developed that could help us understand this variation.

3.3 Use of mutants as genetic stocks

Genetic stocks, broadly defined as plants or populations generated and/or selected for genetic studies, represent a unique and growing class of extremely valuable germplasm which, depending on crop, type of genetic stock and user community, may represent genetic resources of either transient or long-lasting value. Genetic stocks can be divided into three general groups: cytological stocks (e.g. chromosome addition/substitution, aneuploids, amphiploids), mutants (e.g. induced/insertion mutants, tilling populations) and germplasm sets (e.g. mapping populations,

Table 37.2. Selection of desirable traits and development of some commercially exploited varieties through somaclonal variation in different horticultural crops (from Krishna *et al.,* 2016).

Species	Common name	Improved characteristic of somaclone	Reference
Malus × *domestica* Borkh.	Apple	Resistance to *Erwinia amylovora*	Chevreau *et al.* (1998)
Musa acuminata L.	Banana	GCTCV clones; resistance to *Fusarium* wilt	Hwang *et al*. (1992); Hwang and Ko (2004)
Rubus fruticosus L.	Blackberry	Thornless var. 'Lincoln Logan'	Hall *et al.* (1986)
Mangifera indica L.	Mango	Resistant to *Colletotrichum gleosporiensis*	Litz *et al.* (1991)
Ipomoea batatas L. lam.	Sweet potato	Tolerant to salinity	Anwar *et al.* (2010)

Fig. 37.4 (a) (top left): FOC TR4 infested test plot. **(b)** (top right): Shorter 'Formosana' (left) and original 'Formosana' (right). **(c)** (bottom): Comparison of GCTCV-218 'Formosana' (left) and parental Giant Cavendish 'Pei Chao' (right) planted in an infested field.

parental lines, reference germplasm). Any genetic stock collection can represent from a few lines to tens of thousands of lines and therefore can potentially offer a challenge, as well as a burden, to gene bank managers from the standpoint of storage and maintenance. Another challenge with genetic stock collections is the rapidly changing technology used to develop new genetic stocks, which may make older collections obsolete. Therefore, the gene bank manager is faced with having to predict the long-term value, and hence the need for long-term maintenance, of any given collection. Despite the contrasting options of long-term value for some collections versus short-term value for others, there is no question that genetic stock collections should be preserved and that the global system, including CGIAR gene banks, needs to play a role in their preservation.

In April 2010, a group of experts met in Bologna, Italy, for a Genetic Stocks Management Workshop organized in the framework of the System-wide Genetic Resources Programme (SGRP) to develop a curator decision tree (Fig. 37.5) and

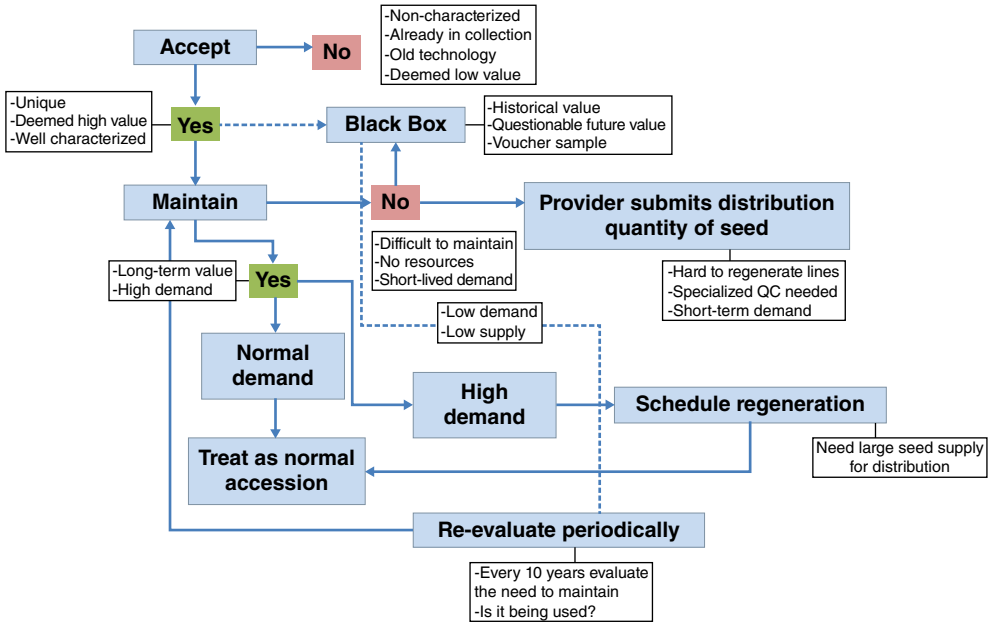

Fig. 37.5. Decision tree offering specific examples for handling genetic stocks in gene banks (SGRP, 2011).

recommendations on the management of genetic stock collections.

Recommendations on genetic stocks (modified from SGRP, 2011) include the following.

- Genetic stock collections are a valuable genetic resource in need of attention from the international community to ensure conservation and access to a wider community.
- An inventory needs to be made of where genetic stock collections are located and who is responsible for the maintenance and distribution of these stock collections.
- There should be involvement of the different crop communities to highlight the urgency of safeguarding genetic stock collections.
- User communities should be a key part of the effort to inventory, collect and safeguard genetic stock collections for target crops.
- A database system is needed which can accommodate data from genetic stock collections.
- There should be regular workshops involving curators and gene bank managers, breeders and researchers to ensure proper identifying, prioritizing and care of genetic stock collections.
- Clear policy rules need to be used when exchanging genetic stocks. Under the Plant Treaty such material can be considered PGRFA (plant genetic resources for food and agriculture) Under Development and is subject to accessibility restriction.
- The international community (CGIAR, FAO, IAEA) should actively support the conservation of genetic stocks of value and importance, as they are tools which can further the mission of sustainably increasing and improving livelihoods.

4 Conclusion

For mass propagation and gene banks wanting to multiply on a large scale and distributing worldwide true-to-type material, somaclonal variants are not desirable and represent a challenge that needs to be overcome using adapted methodologies. Thanks to the sequencing of a great number of crops since the beginning of this century, early detection pipelines could help gene banks avoid propagating or distributing off-types and could improve our understanding of the causes of somaclonal variation occurrence.

Nevertheless, somaclonal variation has proved to be an interesting source of variation and has been used as a genetic improvement methodology, often considered similar to mutation breeding, although no physical or chemical mutagens are believed to be involved. However, our knowledge on the cause of somaclonal variation remains incomplete. As mutants, somaclonal variants even if not directly used as improved germplasm can be used as genetic stocks to finally understand the pathway of many traits, such as the resistance to FOC TR4, as obtained by TBRI on their 'Giant Cavendish' tissue-culture variant (GCTCV) clones through somaclonal variation.

The Joint FAO/IAEA Division with its laboratories and facilities at Seibersdorf, Austria, and its Mutant Variety Database with information on more than 3000 plant mutant varieties (cultivars) is in an ideal position to coordinate the collection of genetic stocks to be distributed worldwide for the benefit of mankind, similar to what the CGIAR centres do within the Genebank Platform.

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