Journal of Applied Biosciences 67:5252 – 5260

ISSN 1997–5902

A review of somaclonal variation in plantain (*Musa spp*): mechanisms and applications

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Original submitted in on 6th May 2013 Published online at www.m.elewa.org on 30th July 2013.

ABSTRACT
Micropropagation or tissue culture is an integral component of applied biotechnology, and is routinely used in different laboratories worldwide. Plant tissue culture has contributed immensely in large-scale production of resistant and pest free plantlets, germplasm exchange and conservation. A common feature of *in vitro*-regenerated plants is the potential of genetic variation generated during tissue culture called somaclonal variation, which can be passed onto progeny.
Objective: This paper reviews the factors responsible for this phenomenon, its occurrence in some crops, with emphasis on banana and plantain and its application in the genetic improvement of *Musa*.
Key words: Plant cell culture, Somaclonal variation, Disease resistance, Banana, Biotechnology

INTRODUCTION:
The accumulation of genetic variability is an important aspect in plant breeding. The increasing use of plant tissue (*in vitro* techniques) as an unconventional means of crop improvement has resulted in the introduction of genetic changes into such plants. These genetic alterations have been recovered in the plants regenerated from cell cultures, and could be used to develop new breeding lines. The occurrence of genetic variation among plants regenerated from *in vitro* culture has been referred to as somaclonal variation (Larkin and Scowcroft, 1981; Ramos Leal *et al*, 1991; Rani and Raina, 2000). Before now, terminologies like calliclones and protoclones had been used to define variants regenerated from culture of geranium (Skirvin and Janick, 1976) and protoplast of potato (Shepard, *et al*. 1980), respectively. However, Larkin and Scowcroft (1981) used the term somaclonal variation for all forms of variation in culture-derived plants. The term 'somaclone' was coined to refer to plants derived from any form of cell culture, somaclonal variation was coined to refer to the genetic variation among such plants. The growth of plant cells *in vitro* and their regeneration into whole plants is an asexual process that involves only mitotic division of the cells. In this context, the occurrence of uncontrolled and random spontaneous variation when culturing plant tissue is a major problem (Leva *et al*.2012). Such variability can involve a single gene mutation at one end of the spectrum, to gross ploidy changes at the other end. Variations have been observed for morphological traits like pigment production, biochemical characters like nicotine synthesis and chromosome number and structure (Evans *et al*. 1984).
Plant tissue culture has been identified as a major aspect of *Musa* germplasm handling (Vuylsteke, 1989). The complex nature of *Musa*- low seed fertility due to triploidy, slow propagation, narrow genetic base, long generation time (10 to 18 months), large

space (6m^2 per plant), has prompted breeders to explore other unconventional means of introducing variability without the need for long-term back-crossing. Somaclonal variation is a hindrance to clonal uniformity, particularly for populations used to establish commercial plantations. However, this phenomenon may also provide a useful source of variation to the plant breeder. Somaclonal variation may be caused by pre-existing variation in the somatic cells of the explant (genetic), variation generation during tissue culture (epigenetic). Factors such as source and age of explants, culture duration, number of sub-cultures, culture environment, chemical additives or growth stimulants or regulators, media composition, the level of ploidy and genetic mosaicism induce variability *in vitro* (Silvarolla,1992). Thus, it is necessary to evaluate its occurrence in some plants and application in the genetic improvement of plantain.

TYPES OF SOMACLONAL VARIATION:

Somaclonal variation results from both pre-existing genetic variation within the explants and the variation induced during the tissue culture phase (Evans *et al,* 1984). There are two types of somaclonal variation: heritable (genetic) and epigenetic. Heritable variation is stable through the sexual cycle or repeated asexual propagation; epigenetic variation may be unstable even when asexually propagated. Epigenetic variation is also known as developmental variation, and includes persistent changes in phenotype that involve the expression of particular genes (Hartmann and Kester, 1983). The best known example of epigenetic variation is the loss of auxin, cytokinin, or vitamin requirements by callus (Jackson and Lyndon, 1990). Other epigenetic changes include, extreme vigour *ex vitro* associated with either the reversion to juvenility (Swartz et al, 1981) or virus elimination (Abo El-Nil and Hilderbradt, 1971). Transient dwarfism is probably epigenetic also, and may be due to a carryover effect of growth regulators from the tissue culture medium.

GENETIC BASIS OF SOMACLONAL VARIATION

Although somaclonal variation has been studied extensively, the mechanisms by which it occurs remain largely either unknown or at the level of theoretical speculation in perennial fruit crops (Leva *et al.* 2012). Few studies have addressed the molecular basis or nature of somaclonal variation (Al-Zahim *et al*. 1999; Yang *et al*. 1999), though it was discovered that alteration in DNA methylation probably plays a role (Muller *et al*. 1990). Prior to the work of Evans and Sharp (1983) to describe somaclonal variation in tomato, the genetic basis of somaclonal variation in asexually propagated crops was not ascertained. It is now understood that for somaclonal variation to be applicable to a wide range of crops, detailed genetic information from the donor crops is necessary. Most of the early works to elucidate the genetic basis of somaclonal variation were done on sugarcane and potato. Both crops are asexually propagated polyploids and can tolerate variation in chromosome number without concomitant disruption in agronomic characteristics. In particular, sugarcane is plastic, and even the intact sugarcane is a chromosomal mosaics (Krishnamurthi, 1981). However, according to Leva *et al*. (2012) the following variations have been observed: changes in chromosome number and structure, in which polyploidy is the most frequent. In addition, single nuclear gene mutations and less-defined genetic changes have been observed. Variation in chromosome numbers and structures, and chromosome irregularities (such as breaks, acentric and centric fragments, ring chromosomes, deletions and inversions) are observed during *in vitro* differentiation and among regenerated somaclones (Hao and Deng 2002; Mujib *et al*. 2007). Such rearrangements in chromosomes may result in the loss of genes or their function, the activation of genes previously silent, and the expression of recessive genes, when they become haploid. The irregularities in the chromosomes may be lost during plant regeneration and result in the production of 'normal' plants, or appear in the regenerated somaclones. Changes in chromosome number are commonly associated with reduced fertility and altered genetic ratios. Chromosome rearrangements have been implicated in the variations in regenerated plants, by analyzing meiosis in regenerated plants. Somaclonal variation may also be due to genetic mitotic recombination, mutations in chloroplast DNA (detected by both maternal inheritance and restriction enzyme analysis). It can also be as a result of the expression of existing genetic differences between the mother plant and the explants, or through the effects induced by the culture media (Tabares *et al.* 1993). In addition, transpositional events, such as the activation of transposable elements, which are pieces of DNA that move within and between

chromosomes, putative silencing of genes and a high frequency of methylation pattern variation among single-copy sequences, play a role in somaclonal variation (Hirochika 1993, Barret *et al*. 2006). The tissue culture environment may result in the modification of DNA methylation patterns (Smulders *et al.* 2011). Global methylation levels and methylation of specific sites are documented in several crops, e.g. oil palm (Jaligot *et al*. 2000), grapevine (Schellenbaum *et al* 2008, Baránek *et al* 2010) and apple (Li *et al.* 2002). In addition, epigenetic changes such as DNA methylation and histone modifications may be associated with the physiological responses of the plant cells to the conditions *in vitro* (Smulders *et al.* 2011). Several epigenetic systems have been studied: variation for morphological traits, such as flower colour and shape, leaf colour and shape, and plant height; resistance to disease; and maturity date (Hammerschlag,1992). The rate of these changes vary not only in response to tissue culture conditions, but also among species and even among cultivars of the same species (Karp, 1982).

Cytoplasmic genetic changes have also been detected by somaclonal variation. The most detailed experiment in this respect was by Gengenbach *et al*. (1977) by evaluating plants for two cytoplasmic traits. Sensitivity to host specific toxin of *Drechslera maydis* race T, the causative agent of southern corn leaf blight, is associated with all genotypes containing Texas male sterility (cms- T) cytoplasm. In seed derived plants, these two characters are tightly linked. Gengenbach *et al* (1977) selected for resistance to toxin and regenerated resistant plants with the aim to recover resistant cms breeding lines, but resistant was associated with a concomitant reversion to male sterility. However, when the restriction endonuclease pattern of mitochondrial DNA (mt DNA) was evaluated, it was evident that significant changes had occurred in mt DNA of plants derived from cell culture (Kemble *et al*. 1982). There was no evidence of comparable changes in chloroplast DNA (cp DNA). Mitotic crossing over also account for some variation observed in regenerated plants, and include symmetric and asymmetric recombination. Mitotic crossing over may account for the recovery of homogenous recessive single gene mutation in some plants (Evans and Sharp, 1983). As breeders have previously only had access to variation that is normally transmitted through meiosis, the recovery of products of mitotic crossing over may constitute a unique source of new genetic variation.

SOMACLONAL VARIATION IN SOME CROPS

Sugarcane (*Saccharum officinarum* L.): Sugarcane is an economically important crop widely cultivated in the tropics to subtropics with an annual production of about 60 to 70 % world sugar (Shah *et al*. 2009).The potential of somaclonal variation for the genetic improvement of characters of agricultural importance was first demonstrated in *Saccharum officinarum* with the *in vitro* selection of a commercial variety resistant to Fiji disease (Heinz, 1973). There were variations in the morphology, cytogenetics and isoenzyme traits. Liu *et al*. (1972) reported on the morphological variation in stooling and erectness amongst somaclones. In addition, some somaclones were reported to be resistant to Fiji disease virus, downy mildew (Krishnamurthi, 1974, Krishnamurthi and Tlaskal, 1974), eyespot disease (Ramos Leal *et al*. 1996) and sugarcane mosaic virus (Nickel and Heinz, 1973). Salt tolerance somaclones have also been generated by a tissue culture cycle (Khan *et al*. 2004). Siddiqui *et al*. (1994) compared the brix % of canes of somaclones with those of their parents and found the somaclones were better than their parents in this character. Conversely, Khan et al. (2004) reported that the brix % of canes of somaclones was found to be less compared to their parents. The somaclones were better in tillers/plant, stalk height, number of nodes/stem and root band width. Evidently, somaclonal variation is very common in sugarcane, and it affects many important traits that can be used in the improvement of some varieties.

Potato (*Solanum tuberosum*): Potato is one of the most important vegetable crops in the world (Solomon-Blackburn and Baker, 2001). Potato is a native of South America (Peru) where considerable breeding programmes have taken place. With over 70% of the world potato grown in Europe, potato is considered a good source of antioxidants (Chen *et al.* 2007). In North America, the more than 70 year old variety called 'Russet Burbank' constitutes about 39% of the potato crop. As a vegetatively propagated, heterozy-gous and tetraploid crop, traditional breeding of potato is very difficult (Solmon-Blackburn and Baker, 2001). The same applies using botanical seeds on commercial cultivation, which is fraught by low germinability and large variability in the segment generations (Bordallo *et al*. 2004). Somaclonal variation has been reported in potato plants regenerated from protoplasts of the widely grown variety 'Russet Burbank' (Bannaceur *et al*. 1991). Statistically significant and stable variations were found for several morphological characters such as compactness of growth habit, date of maturity, tuber uniformity and skin colour of

the tubers. Some of the somaclones also had greater resistance to *Alternaria solani* toxin than the parents; while others were resistant to late blight caused by *Phytophthora infestans*. These somaclones were stable through a number of vegetative generations. Wenzel (1979) observed phenotypic variability from protoplast-derived somaclones of potato diploids after extended periods in culture, which he attributed to culture-induced aneuploidy. Somaclonal variation was used to select potato calli with desirable traits, such as salt tolerance and drought stress (Ehsanpour *et al*. 2007). Recently, Khatab and Antar (2011) employed UV-C radiation to induce somaclonal variation in potato callus cultivar 'Cosima' and its detection using RAPD-PCR. Rosenberg *et al* (2010) investigated the effect of thermotherapy on the new potato variety Reet clones which differed in yield, number and weight of tubers, late blight resistance and morphological characteristics. Thus, somaclonal variation has for long been used to improve potato cultivars.

Rice (*Oryza sativum*) : Somaclonal variation on rice is particularly interesting because apart from being a model plant for the grass family (Poaceae) that includes all cereal crops, it is also a major crop that provides food for more than half of the world's population (Ngezahayo *et al*. 2007). Several studies on somaclonal variation have been carried out in rice by using cultivars of both subspecies: *indica* and *japonica* (Yang *et al*. 1999; Kim *et al*. 2003; Roy and Mandal 2005). Ngezahayo1 *et al*. (2007) studied the nature of somaclonal variation at the nucleotide sequence level in the cultivar rice Nipponbare using RAPD and ISSR markers and by pairwise sequence analysis. Earlier reports on somaclonal variation in rice include those of Nishi *et al*. (1968) and Henke *et al*. (1978), all from rice callus. Variations were observed in number of tillers per plant, number of fertile tillers per plant, length of panicle, frequency of fertile seeds, plant stature and length of flag leaf. Oono (1978a and b) extensively and carefully analyzed homozygous maternal seeds from selfed double haploids of about 800 somaclones derived from callus. After two selfing generations, all the lines were examined for chloroplast content, flowering date, plant height, fertility and morphology with about 28.1% as true-to-types (normal parents). Variations were however observed in seed fertility, plant height and heading date. There were variations in chlorophyll deficiencies in the second generation. Sectorial analysis of plants derived from a single seed callus showed that at least most the variations were induced during culture.

Maize *(Zea mays):* Maize is a very important crop used as food for man and livestock. It is also used in many industrial products such as textiles, ceramics and pharmaceuticals (Earle and Kuehnle, 1990). Moreover, maize has many other features that make it attractive material for studies of somaclonal variation. Matheka *et al* (2008) used somaclonal variation to select maize varieties resistant to drought in Kenya. Somaclonal variation in maize has also been shown to affect the mitochondrial genome. Selection for resistance in cultures of T-cytoplasm maize (sensitive to southern corn leaf blight T-toxin of *Drechslera maydis* Race T) by recurrent sub-lethal exposure T toxin resulted in the recovery of toxin-resistant plants. These same plants were also fertile in contrast to the male-sterility of the original parent (Gengenbach *et al*. 1977). These results have been confirmed by Brettell and Ingram (1979) and Brettell *et al.* (1980). They further indicated that the frequency of occurrence of these resistant variants was very high even when the toxin was not added to the cultures prior to regeneration. The restored male-sterility and toxin resistance were shown to be cytoplasmically inherited.

SOMACLONAL VARIATION IN PLANTAIN (*MUSA SPP.)*

Occurrence: Somaclonal variation is a common phenomenon in both *in vitro* and *in vivo* propagated *Musa* plants. Such somaclones (off-types) are more common in bananas and plantain regenerated in vitro than in conventionally propagated *Musa* (Drew and Smith, 1990; Smith, 1988; Vuylsteke *et al*. 1988; Vuylsteke and Ortiz, 1996). Agronomically, micropropagated bananas and plantains are capable of performing as well as, if not better than plants derived from conventional plants (Drew and Smith, 1990). Certain genotypes are known to exhibit a high variation rate, while others rarely do (Cote *et al*. 1993; Smith, 1988; Vuylsteke *et al*. 1991). Dwarfism in the 'Cavendish' bananas and inflorescence variation in the plantains are the most commonly observed morphological changes. Changes in leaf size, shape and colour of the pseudostem and flower are also common. In micropropagated 'False Horn' plantains, variation in inflorescence type in the form of reversion to a typical 'French' plantain bunch type accounts for 40-100% of the total variability (Vuylsteke *et al*. 1988). In addition, the frequency of this variability is over-amplified principally because, the off-type is not detected in culture and the numbers are multiplied by repeated sub-culturing of the variant line. Smith (1988) reviewed the factors influencing somaclonal variation in bananas and divided them into intrinsic and culture-induced factors. Genetic changes

induced during micropropagation can be influenced by the choice of explants, the composition of the media (nature and concentration of phytohormones), number of subcultures or length of time of in culture, and the level of dedifferentiation the tissues undergo in the culture. Intrinsic factors include the genetic stability of the cultivar or genotype used in micropropagation. Some works have been done on the characterization of somaclonal variation in plantain (Krikorian *et al*, 1993; Nwauzoma, 1999; Nwauzoma *et al*. 2002). In addition, *Vuylsteke et al*. (1996) gave a comprehensive report on the agronomic performance of some somaclones obtained from the 'False Horn' plantain cultivar. The variants differed from the true-to-types in inflorescence morphology, as well as in quantitative characters like fruit maturity, leaf size, yield and yield components. Only the 'French reversion' variant, which resembled an existing cultivar, out-yielded the true-to-type. However, its fruit weight and size, which affect consumers' preference, were lower.

Use in breeding strategies: Plant breeding involves two main phases- the creation of genetic variability and the selection for improved gene combination among variants (Evans *et al*. 1984). To have an impact on breeding schemes, an *in vitro* system must produce a plant (variant) useful for breeding. A few somaclones have been released as named cultivars (Skirvin *et al*, 1994). Ostry and Skilling (1987) screened poplar regenerants with *Septoria musiva* and isolated stable somaclones with putative resistance to this fungus. A comprehensive study on the potential of somaclonal variation in the genetic improvement of plantain has been reported by Nwauzoma *et al* (2002). Approximately 500 somaclones each from 'Agbagba' ('False Horn' plantain) and 'Bise Egome' (French plantain) were field evaluated for their agronomic performance and response to black Sigatoka disease caused by *Mycosphaerella fijiensis*. The micropropagated populations were independently generated from a number suckers from each accession. There were significant differences between micropropagated accessions and the different crop cycles. Differences between plants derived from suckers of the same accession were also expressed, indicating the chimeric nature of variation in the traits examined, as earlier reported (Newbury *et al*. 2000). That many *Musa* accessions might be chimeras would explain how *Musa* landraces, such as 'Bise Egome' and 'Kluai Tiparot', can exhibit a regular alternation of bunch morphology between successive sucker generations, despite this character having a complex genetic basis (Ortiz and Vuylsteke, 1998). Genetic transformation and somaclonal variation have been used to incorporate or identify durable disease resistance in *Musa* (Sagi *et al*. 1997; Crouch *et al*. 1998; Nwauzoma *et al*. 2002 and 2004). Hwang and Ko (1987), identified somaclonal variants of the cultivar 'Giant Cavendish' with putative field resistance to Fusarium wilt (race 4), but all had inferior horticultural characteristics, including poor yield and low fruit quality. The variants were mostly inferior to the 'Cavendish' clone from which they were derived. However, further evaluation among in vitro or sucker-derived clones of these resistant variants resulted in the identification of plants with improved traits when compared to the original variant, yet slightly lower-yielding than the true-to-type cultivar (Hwang, 1988; Hwang *et al*. 1993). Also there was a breakdown in resistance to *Fusarium* wilt, when they were tested at other locations. This observation may be due to problems in screening for this disease, the occurrence of genotype-by-environment interaction for this trait or genetic diversity of the pathogen (Israeli *et al*. 1995). These examples suggest that somaclonal variation from micropropagated banana may increase the level of resistance existing in susceptible cultivars. Thus, somaclonal variation may produce useful lines with tolerance to major diseases. The power of somaclonal variation for plant improvement is that new traits are developed in the best available cultivars. Most scientists perform somaclonal variation research with elite clones, potential cultivars or breeding lines. The variation observed in such materials can result from various sources, including the activation of previously silenced genes present in the genome.

CONCLUSION

The relevance of micropropagation rests on the recovery of disease and pest resistant materials in culture. Literature is replete with the occurrence of variability among *in vitro* generated plants from cell culture. Somaclonal variation is wide spread, being found not only in asexually propagated crops, but also in seed propagated and self-fertilizing species. Possible causes of somaclonal variation include pre-existing differences in the somatic cells of the explants and those generated in culture. The development and application of molecular and genetic techniques and sophisticated manipulation of cultured plant cells will doubtless identify and characterize specific genetic mechanisms. One of the goals of somaclonal trials is to identify somaclones that can be used directly or form basis for breeding. Already, somaclonal variation has some impact on the

improvement of sugarcane, maize, plantain and potato, and in the breeding of new floricultural varieties. Somaclonal variation may find its greatest application for plant improvement in concert with selection for desirable mutations at the cellular level if it interfaces with conventional breeding. Potentially useful somaclones should be evaluated under relatively stringent field conditions to confirm their agronomic, disease resistant or breeding values.

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