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Review

Somaclonal variation associated with oil palm (*Elaeis guineensis* Jacq.) clonal propagation: A review

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Somaclonal variation refers to any phenotypic or genotypic modifications that arise from *in vitro* culture. In the oil palm, it is characterized by fruit mantling and abnormal vegetative growth. Tissue culture remains the only means of micro propagation of oil palm as its biological characteristics do not allow for vegetative propagation by conventional means. The early success of plantlets production inspired many oil palm organizations to explore *in vitro* propagation technique. Though oil palm tissue culture is already well established, it is still faced with many challenges. Prominent among them is somaclonal variation which was first reported in 1986. They are only detectable when the palms start flowering; that is, after two to three years in the field. It has not been possible to fully eliminate or circumvent floral abnormality in the oil palm. However, the adoption of several measures such as reducing hormone level, avoiding fast growing callus and, reducing culture period, have reduced the problem to manageable levels of < 5%. Possible causes and factors influencing somaclonal variation in the oil palm are discussed.

Key words: Somaclonal, variation, propagation, *Elaeis guineesis,* ramets, embryogenesis.

INTRODUCTION

Somaclonal variation is a genotypic or phenotypic modification due to a variety of causes resulting in variation of the progeny of clonal propagation. This means a significant percentage of the regenerated plants may not be identical in genotype and phenotype to the plant from which the original explants were obtained (Evans et al., 2003). Simply put, somaclonal variation is mutation that occurs in tissue culture. It is not restricted to but is particularly common in plants regenerated from callus such as the oil palm. The oil palm, *Elaeis guineensis* Jacq, belongs to the Family Arecaceae. It is a perennial monocot and monoecious, that is, male and female flowers occur separately on the same plant, usually in distinct male and female inflorescences (Corley and Tinker, 2003). It is one of the most efficient oil bearing crops in the world with average yield of 4 to 5 tons of crude oil per hectare and up to 7 to 8 tons of crude oil per hectare (Te-Chato and Hilae, 2007). The oil palm produces seven times more vegetable oil per hectare than soybean which is the largest source of edible vegetable oil in the world (Ndon, 2006). The major centre of oil palm production is in South East Asia with Malaysia and Indonesia together accounting for around 83% of world palm oil production in 2001 (Wahid et al., 2004).

The oil palm is an important economic crop, producing food and raw materials for the food, confectionary, cosmetics and oleo-chemical industrial demands of oil palm products. In the past, planting material consisted solely of seed-derived and mainly of the *Tenera* hybrids (fruit with shell of intermediate thickness) originally from crosses between *Dura* (thick shell) and *Pisifera* (thin

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shell) types (Rival, 2000). Conventional plant breeding techniques have also been used to develop, improve and conserve elite genotypes. However, since each selection cycle lasts for around 10 years, genetic improvement is inherently very slow, and high heterogeneity is still observed among hybrids. When these characteristics are joined with the low planting density (generally 143 palms/ha) and the necessity of establishing seed orchards for the production of commercial material, it can be seen that oil palm improvement is labour intensive, time consuming and therefore expensive (Rival, 2000). The biological characteristics of the oil palm do not allow its vegetative propagation by conventional horticultural means such as cutting, bud grafting, suckers etc. It has a single growing point and does not produce offshoots like some other palm species as all the auxillary buds form inflorescences and there is no reported method for the establishment of cutting (Vovola and Lord, 2004).

These constraints which exist in oil palm breeding make desirable the development of a micropropagation technique such as tissue culture which is an efficient and effective method for rapid multiplication of uniform planting material with high genetic potential.

BACKGROUND OF OIL PALM TISSUE CULTURE

The tissue culture technique for oil palm was developed in the 1970s (Jones, 1974; Rabechault and Martin, 1976). The oil palm industry was quick to capitalize and commercialize this new technology as the clones hold a promise of an estimated 30% increase in yield compared to commercial seedlings (Hardon et al., 1987). One possible way to clonally propagate elite oil palm is by means of somatic embryogenesis on calli derived from various origins (Rival, 2000). The early success of plantlet production in the 1970s and increased yield potentials inspired many oil palm organizations to exploit the *in vitro* propagation techniques. Most of the laboratories involved in the oil palm industry now have well established tissue culture media and protocols. However, the large number of variables in the culture process has meant that different laboratories often obtain different results from superficially similar treatments. Even in the same laboratory, results are not always reproducible, so there remains some uncertainty as to the best procedure (Corley and Tinker, 2003). To date, whole plants have been successfully regenerated from various explants and callus derived from seedlings of the oil palm. They include mature and immature embryos, apical meristems, embryogenic cells, suspension cultures, friable, embryogenic tissues, roots, inflorescence and young leaves (Teixeira et al., 1993, 1994). However, in 1986, problem of clonal fidelity associated with somaclonal variation was reported (Corley et al., 1986).

It was noticed that some of the clones planted were not flowering normally, instead had a high incidence of flowers with 'mantled' character (conversion of stamen whorl into a carpel whorl). This led to the destruction of several hectares of clonal palms. Many laboratories reduced production, but maintained enough for field evaluation. Somaclonal variation has till date impeded commercial large-scale production of oil palm planting material via *in vitro* culture. The somaclonal variant can exhibit a marked heterogeneity in its occurrence and intensity between different clone lines, between palms of the same line, and between flowers of the same individual variant palm (Jaligot et al., 2000). With concerted research efforts in various tissue culture laboratories in the world, more information and understanding on the tissue culture process and the problems arising from it have accumulated. It is therefore hoped, that floral abnormality in oil palm may soon be overcome or circumvented. This review seeks to address the origins and mechanisms of somaclonal variation, its causes and factors influencing it; the phenomena in oil palm clones, symptoms and problems associated with it as well as control measures.

ORIGINS AND MECHANISMS OF SOMACLONAL VARIATION

According to Evans et al. (2003) and Morcillo et al. (2006), there can be two major classes of somaclonal variation: genetic and epigenetic variations:

Genetic (heritable) variability is caused by mutation or other changes in DNA such as changes in chromosome number and structure. Various molecular mechanisms are responsible for genetic variability associated with somaclonal variation.

Changes in ploidy level

This refers to changes in chromosome number originating from abnormalities that occur during mitosis for example, aneuploidy (having more or less than an integral multiple of the haploid number of chromosome), polyploidy [having three or more times the haploid (n) number of chromosomes] and mixoploidy (the presence of cell lines with a different genetic constitution in an individual).

Structural changes in nuclear DNA

This appears to be a major cause of somaclonal variation. The change can modify large regions of a chromosome and so may affect one or several genes at a time. Such modifications include: deletion (loss of gene), inversion (alteration of gene order), duplication (duplication of genes), translocation (segments of chromosomes

moving to new locations), activation of transposons (transposable elements) and point mutation (chance rearrangement of the four bases) in the DNA structure.

Epigenetic (non- heritable) variability

This is stably transmitted modifications that result in stable phenotype differences even when the parent cells are genetically identical. Epigenetic changes are often temporary and plants may revert to the normal phenotype relatively easily, but some can be long lasting and may even be transferred during sexual propagation (Smulders and De Klerk, 2011). Epigenetic variability maybe caused by DNA methylation, DNA amplification, histone modification and activation of transposable elements (transposons). These modifications may influence gene transcription.

One major characteristic difference bet-ween genetic and epigenetic changes is that, whereas genetic changes occur at random, same epigenetic changes can be 'reproduced' when same condition are imposed during the production of another population (Smulders et al., 1995; Smulders and De Klerk, 2011).

SOMACLONAL VARIATION IN OIL PALM CLONES

Oil palm clonal propagation though successful, has not been without some difficulties. Problems with clonal fidelity associated with somaclonal variation have been encountered. Somaclonal variation, specifically in the form of flower and fruit mantling, has impeded commercial large-scale production of oil palm planting materials through tissue culture. According to Kushairi et al. (2010), the first case of abnormality in clonal palm was brought to the attention of a conference organized by the International Society for Oil Palm Breeders (ISOPB) in 1985 and later reported by Corley et al. (1986). It was observed that some clones planted were not flowering normally, instead had a high incidence of flowers with 'mantled' character (rudimentary stamen primordial on female flowers developed into supplementary carpels). That is, feminisation of the male parts in flowers of both sexes. This abnormality is accompanied by parthenocarpic (seedless) fruit set and severe bunch failure. Approximately, 5% of somatic embryo-derived palms show abnormalities in their floral development (Corley et al., 2003; Jaligot et al., 2000; Rival, 2000). The somaclonal variant can exhibit a marked heterogeneity in occurrence and intensity between different clone lines, between palm of the same clone line, and between flowers of the same individual variant palm.

Interestingly, reversions to the normal phenotype over time have been found to occur, leading to a complete recovery of the normal phenotype for 100% of the slightly 'mantled' individuals, and for 50% of the severely ''mantled'' ones after nine years in the field (Jaligot et al., 2000).

Causes and factors influencing somaclonal variation in oil palm

The causes of clonal abnormality in oil palm remain unknown but experimental evidence suggests that the floral abnormality observed in clonal palm may often be epigenetic in nature although genetic causes cannot be ruled out (Alwee et al., 2001). However, the following have been implicated in the problem.

Genotype of explants

Genotypic differences exist in oil palm and this can confer susceptibility/tolerance to the abnormality (Soh et al., 2011; Eeuwens et al., 2002).

Hormone type and concentration

These have been associated with somaclonal variation especially high levels of the chlorinated auxin 2,4-dichlorophenoxy acetic acid (2,4-D), reported to cause mitotic spindle abnormality (Duval et al., 1988; Sogeke, 1998).

Length of culture period

The longer the cells that remains in culture, the greater their chromosomal instability. Prolonged culture period *in vitro*, especially the callus stage shows a higher frequency and degree of abnormality (Corley et al., 1986; Rohani et al., 2003).

Use/type of callus in culture

In vitro systems that uses callus have been found to produce aberrant clones the most. Nodular compact calli have been found to produce on the average, 5% variant palms with fast growing calli producing up to 100% (Jaligot et al., 2000).

In vitro culture regime

The composition of the growth medium can trigger changes in ploidy and cultures grown under nutrient limitations can develop abnormalities. Tissue culture media can change the level of DNA methylation and thus maybe one of the important causes of somaclonal variation (Evans et al., 2003; Morcillo et al., 2006).

Table 1. Estimated world production of oil palm tissue culture plantlets.

Country	Million ramets/year
Malaysia	2.5
Costa Rica	0.5
Indonesia	0.5
Total	3.5

Source: Kushairi et al. (2010).

Type of regenerative process (organogenesis or embryogenesis)

The oil palm currently uses the indirect somatic embryogenesis method and most somatic embryo-derived palms show abnormality in their floral development (Armstrong and Phillips, 1988). The frequency of somaclonal variation in regenerated plants depends on the genotype of the material, type of explants used, age of culture, type of regenerative process (organogenesis or embryogenesis), stress inherent in cellular deprogramming induced by plant growth regulators such as the synthetic auxin 2,4-dichlorophenoxy-acetic acid (2,4-D) (Morcillo et al., 2006). Other stress conditions could be water deficiency, osmotic stress, heat, extreme procedure in tissue culture, nutrient media conditions etc. The age of explants source also influence somaclonal variation as the frequency of changes in ploidy increases with age of plant cells and therefore in chromosomal instability (Evans et al., 2003; Smulders and De Klerk, 2011).

WORLD PRODUCTION STATUS OF OIL PALM PLANTLETS

About 20 oil palm laboratories with varying capacities are in operation throughout the world. According to Kushairi et al. (2010), it is estimated that there is a ready market for more than 100 million tissue culture plantlets annually based on current demand for oil palm seeds in Malaysia and other countries. Some five million oil palm plantlets are currently being produced annually worldwide (Table 1) with Malaysia alone contributing over 80% of the total production from 11 commercial tissue culture laboratories such as Advanced Agric-ecological Research (AAR) and Federal Land Development Agency (FELDA).

Oil palm clonal abnormality is epigenetic?

Experimental evidences abound that suggest or point to the epigenetic nature of oil palm clonal abnormality.

1) Reversion of abnormal palms in the field over a period of years (Jaligot et al., 2000; Morcillo et al., 2006).
2) Non-Mendelian transmission of the trait by convention genetic crossing that is, inherited in non-Mendelian fashion (Morcillo et al., 2006).
3) Absence of any detectable defect in DNA organization (Jaligot et al., 2000).

Plate 1. Normal oil palm fruits. Source: Soh et al. (2011).

Symptoms and problems associated with oil palm clonal variation

The oil palm clonal abnormalities are only detectable once palm start flowering; that is, after two to three years in the plantation. Therefore, the cost, time and labour spent on production constitute huge losses. The abnormality is characterized by:

1) Flowers with ‘‘mantled’’ character (Plates 1 and 2); that is, flowers develop a secondary carpel whorl instead of stamen (apparent feminization of male parts in flowers of both sexes) (Corley et al., 1986; Alwee et al., 2006; Jaligot et al., 2000).
2) Parthenocarpic (seedless) fruit set, small bunches and severe bunch failure, Plates 3 and 4 (Corley et al., 1986; Rival, 2000).
3) Partial or complete flower sterility, poor pollination, extended juvenility (Plates 5 and 6), fruit abortion and therefore no yield (Soh et al., 2011; Morcillo et al., 2006).
4) Abnormal vegetative growth (Plate 7 and Table 2) (Guzman and Peralta, 2010).

Control measures to floral abnormality

It has not been possible to fully eliminate or circumvent floral abnormalities in oil palm clones. However, the adoptions of the following measures have kept it to manageable levels of less than 5%.

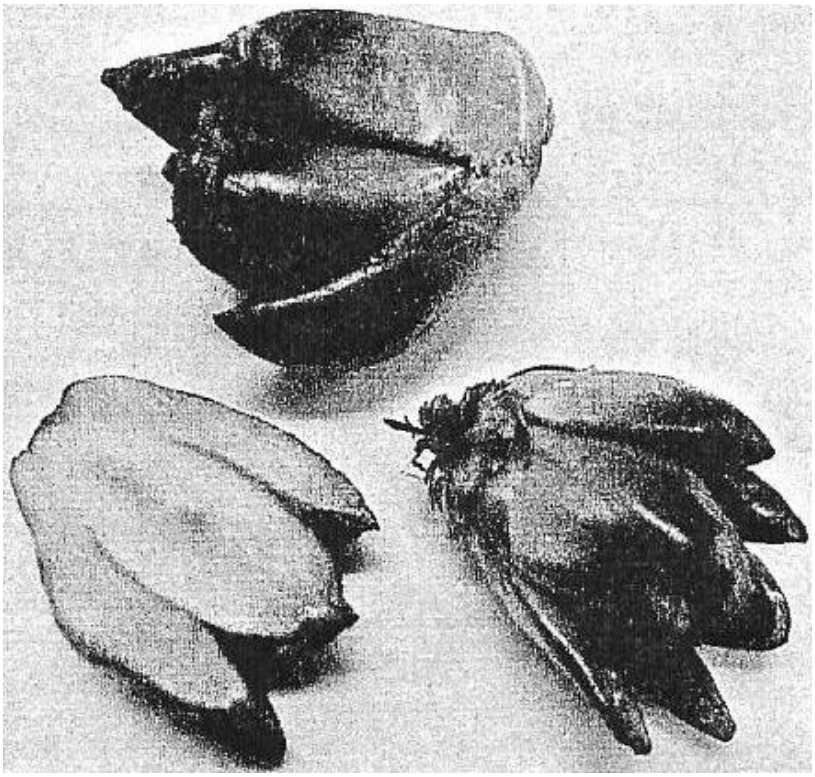

Plate 2. 'Mantled' oil palm fruits. Source: Soh et al. (2011).

Plate 3. (a and b) Normal and abnormal bunch.Source: Smulders and De klerk (2011).

1) Reducing hormone levels or total avoidance of them in certain culture stages (Duval et al., 1988).

2) Replacing chlorinated auxin, 2,4-D with non-chlorinated auxin, NAA (Sogeke, 1998).

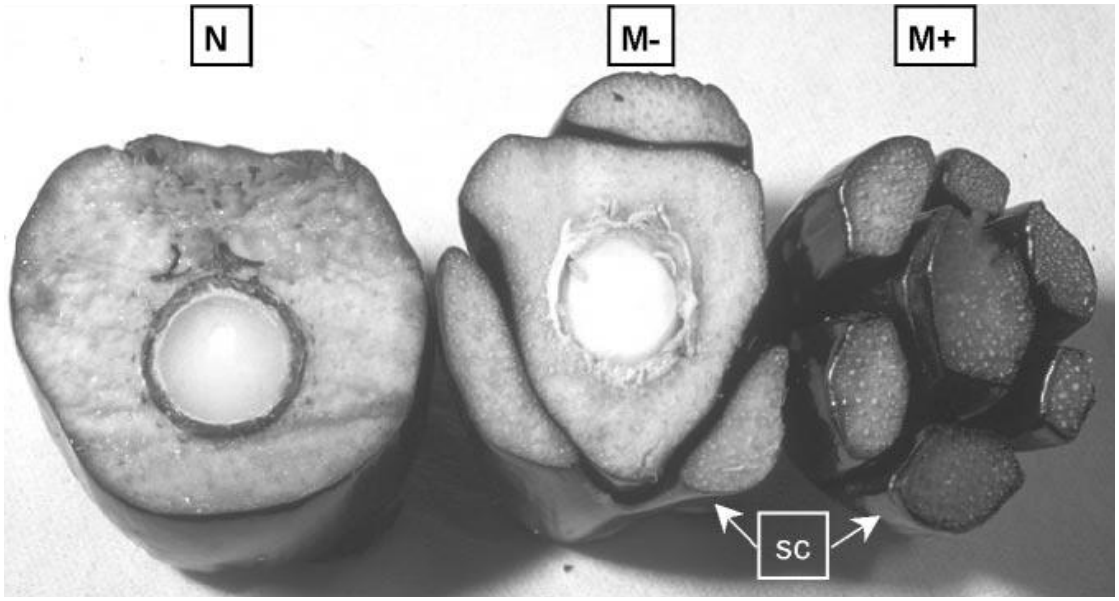

Plate 4. Cross-section of oil palm fruits originating from somaclones. N, fruit from normal; M, slightly mantled; M+, severely mantled; SC, supplementary carpel. Source: Jaligot et al. (2000).

Plate 5. Somaclonal oil palm with extended juvenility.Source: Soh et al. (2011).

Plate 6. Oil palm tree with normal fruits and leaves fruits/leaves. Source: Soh et al. (2011).

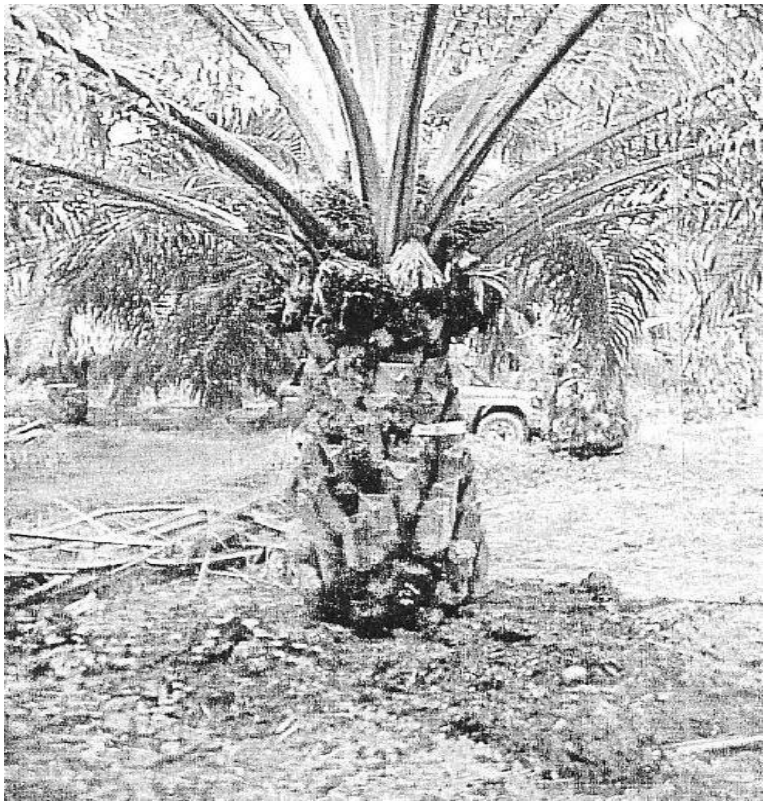

Plate 7. Oil palm with abnormal vegetative growth. Source: Soh et al. (2011).

Table 2. Comparison of normal and abnormal ramets.

Normal ramets	Abnormal ramets
Successive leaves in a shoot differ in shape.	Successive leaves are similar in shape
Lower (older) leaves are smaller than newly formed, expanded leaves	Older and new leaves attain similar size
Soft and fibrous texture of leaves	Hard and plastic-like texture of leaves.
Newly, expanded leaves with a prominent angle with respect to the vertical	Newly, expanded leaves with little or no angle with respect to the vertical
Pale green new leaves	Dark green new leaves

Source: Guzman and Peralta (2010).

3) Reducing culture period by reducing the number of subculture cycles and number of plantlets produced. Discard after about two years (Rohani et al., 2003).
4) Avoid fast-growing callus as high multiplication rates magnify any problem (Jaligot et al., 2000; Smith et al., 2010).
5) Stringent selection/strict culling at the earliest stages as a problem may not appear until after plants have been grown in permanent location (Kushairi et al., 2010; Guzman and Peralta, 2010).
6) Practicing strict process quality control (low contamination, optimum growing condition, stringent culture selection at each transfer (Soh et al., 2011).
7) Developing diagnostic tools for predicting genetic predisposition to abnormality. These include global gene expression analysis via DNA microarray, genetic mapping and candidate gene approach (Wahid et al., 2004; Morcillo et al., 2006).
8) Cloning a large pool of selected palms with different genetic background (genotypes) as there are genetic/clonal differences in susceptibility/tolerance to the mantling abnormality (Soh et al., 2011).
9) Direct organogenesis to by-pass the callus stage but this is not yet feasible in the oil palm though direct or 'true' somatic embryogenesis has been reported (Smith et al., 2010).

CONCLUSION

The oil palm is an important economic crop and the most efficient oil-bearing plant in the world. Due to increasing demand for oil palm planting materials and products coupled with the slow conventional breeding methods for its improvement, there is need for alternative means for improvement of elite hybrids. As oil palm has no known method of conventional vegetative propagation like other crops, clonal micropropagation becomes a viable means of rapid multiplication of elite genotype with desirable characteristics. The world production of oil palm tissue culture plantlets is about five million with Malaysia contributing above 80%; although, oil palm tissue culture has been successful but not without some difficulties. Major among these difficulties is somaclonal variation (epigenetic) expressed as vegetative and floral abnormalities which was first reported in 1986. This caused a major setback in the industry leading to the destruction of hundreds of hectares of oil palm plantation worldwide. Though these abnormalities still exist but at minimal and manageable levels of less than 5%. It is hoped that with current myriads of research and developmental efforts going on in various laboratories in the world to further resolve or circumvent the amenability and fidelity deficiencies in tissue culture, clonal palms are expected to eventually replace seed-derived planting materials on a commercial scale.

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