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Somaclonal variation in fruits

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Abstract
In pomology many advancements have been made in the sector of micropropagation which is widely used now a days for commercial propagation of many fruit crops. Micropropagation ensures preservation of true to type character from generation to generation and can be used for preserving some superior characteristics and uniformity among regenerated plants. However, propagation by micro propagation and tissue culture method can results in variations which can be genetic or morphological i.e., somaclonal variations. The current review discusses about somaclonal variation, how somaclonal variation occur in plants, different factors which influences the somaclonal variation, advantages and disadvantages of somaclonal variation and a case study on somaclonal variation in Olive.

Keywords: Somaclones, gene amplification, micro propagation, crop improvement

Introduction
In order to describe the genetic diversity that can occur in plants that have been cultivated from any kind of cell culture, the term "Somaclonal Variation" was developed (Larkin & Scowcroft, 1981) [37]. Somaclonal variation is phenotypic diversity that occurs among somaclones and can be genetic or epigenetic in origin. Somaclonal diversity evolves as a result of tissue culture phase variation and pre-existing genetic variation within the explant (Evans *et al*., 1984) [16]. Numerous plant species have been found to exhibit variation in somaclones with respect to karyotype, isoenzyme pattern, precocity for bearing, ploidy level, growth, yield, quality, pigmentation, disease resistance, and resilience to hard soil and climatic conditions. (Patil and Navale, 2000) [59].

In the early days of micropropagation, it was established as a dogma that all plants originating from tissue culture should be an exact copy of the plant that served as their parental source. However, phenotypic variants were frequently observed in plants that had been regenerated. In most cases, plants that had been affected by variation were thrown away. Sometimes it was thought that the variant plant arose as a result of recent exposure to exogenous phytohormones, and other times they were labelled as a "epigenetic" occurrence, which rendered them unworthy of further scientific study. Both of these explanations were based on the assumption that the variant plant had recently been exposed to the exogenous phytohormones.

The term "Calliclone" refers to plants that have successfully reproduced from a stem callus (Skirvin and Janick, 1967), while plants that regenerated from the leaf are known as 'Protoclones' (Shepard *et al*., 1980) [74]. Larkin and Scowcroft (1981) [38] coined the term "Somaclones" to describe plants generated from any type of cell culture. The variance seen among such plants is referred to as 'Somaclonal Variation.'

It was expected in the early days of the application of tissue culture that the plants grown would be clonal; however, this is no longer the case. It is common practise to produce 'off type' plants, which have been determined to be genetic mutants that were developed during mitotic processes in the process of tissue culture and are mutations that pass on to following sexual reproduction cycles. (Sahijram *et al*., 2003) [69]. All aspects of *in vitro* produced plants, including their undifferentiated cells, isolated protoplasts, calli, tissues, and morphological characteristics, are susceptible to genetic modification (Currais *et al*. 2013) [9]. On the other hand, neither the stability nor the heritability of any variation that results via micro-propagation can be guaranteed. Because of their epigenetic nature, some differences are not always passed on from parent to child. The most significant obstacle that has been presented to the broad application of micropropagation and other tissue culture methods in agricultural crops is the phenomenon known as somaclonal variation (Peschke and Phillip, 1992) [62].

On the other hand, particular somaclonal variants have been found to be of agronomic and commercial value, and in a few instances, they have even been released as new cultivars.

Cultures of plant cells and tissues produce a greater genetic diversity in a shorter amount of time and with less complicated apparatus.

This technology has a lot of potential in crop improvement for horticultural crops that are mostly propagated vegetatively. Some of the factors that contribute to this potential include: a longer juvenile phase in perennial fruit crops; occasional inbreeding depression; self and cross incompatibility; a small genetic base in ornamentals; and other similar factors. Additionally, in contrast to the cross seedlings of perennial crops, which demand a significant amount of area and time for *in vitro* screening of desirable features, somaclonal variations call for less space and less time. Plant breeding and genetic enhancements may benefit from the use of somaclones, and the recovery of novel variations may be facilitated through the use of sufficient *in vitro* selection pressure. (Jain 2001; Lestari 2006) [32, 40].

Plants that have been micro-propagated are currently favoured over plants that have been conventionally propagated in a variety of crops, including strawberries, papaya, bananas, grapes, pineapples, citrus fruits, tomatoes, cucumbers, watermelons, rhododendrons, and orchids, amongst others. Since 1971, when the first documented report of morphological differences in *in vitro* sugarcane plants was published (Heinze and Mee 1971) [24]. Some of the potential improvements that have been discussed include enhanced resistance to diseases caused by fungi, bacteria, and viruses; enhanced resistance to insects and worms; increased economic output; and enhanced tolerance to drought, cold, salt, and aluminium.

Somaclonal variation arose from

Karyotype changes

Previously, it was commonly considered that somaclone variants were the result of substantial karyotype abnormalities like as aneuploidy or polyploidy (Murashige 1974; Thomas *et al*., 1979) [55, 82]. Indeed, gross karyotype changes have been reported often in tissue grown plant cells (Murashige and Nakano 1967) [56].

Changes in the cryptic Linked to Chromosome Rearrangement

More subtle chromosomal rearrangements, rather than dramatic alterations in chromosome counts, may be responsible for genetic variation in cultured cells.

Loss of genetic material due to chromosome rearrangement might result in phenotypic variations. In addition to disrupting the gene where the chromosomal break occurs, neighbouring genes, particularly those whose transcription may be coordinatedly controlled, would be affected.

This phenomenon is referred to as the "Position effect," and it has been extensively studied and documented in *Drosophila* (Lewis 1950; Spofford 1976) [42, 78]. Additionally, it is hypothesised that the Position effect occurs in plants such as Oenothera (Catcheside 1947) [7].

The expression of genes that were dormant before can be a consequence of cryptic modifications, which can also result in the loss of genes and the activities those genes once performed. It is possible, for instance, for an arrangement to get rid of or in some other way turn off a dominant gene, which then enables the recessive allele to change the phenotype. The term "culture induced hemizygosity" was used by Siminovitch to describe this situation (1976).

Transposable Elements

Numerous investigations have led to the conclusion that transpositional events might be responsible for some of the somaclonal variation that occurs. There is a possibility that the environment of the tissue culture is especially favourable to DNA sequence transposition. Somatic Darwinism was coined by Weill and Reynaud (1980) [88] to describe subsequent adaptiveness in somatic tissue. Doolittle and Sapienza (1980) [13] contended that transposable elements and certain classes of intermediate repetitive DNA may be ubiquitous, and that their primary role is to ensure their own survival in the genome, therefore dubbed selfish DNA.

Variability induced by sequence transposition is an excellent method of increasing adaptability to novel environments, such as that found in cell culture. Such putatively created variations have the potential to be integrated into germ line cells in plants.

Gene Amplification

It has recently come to light that some genes in higher plants are able to amplify themselves throughout the process of differentiation or in reaction to environmental stressors. This amplification leads to an increase in the number of copies of that gene that are present in each haploid genome. Depending on the mechanisms that regulate gene expression, this could suggest that the amount of mRNA and protein that is produced by that gene is increased (Schimke *et al*. 1977) [71]. Amplification or depletion of DNA sequence copies can also occur in plant cell cultures, which may account for some of the somaclonal diversity.

Somatic Crossing Over and Sister Chromatid Exchange:

Asymmetric somatic cell sister chromatid exchange can result in genetic material loss and duplication. The frequency of variation in the frequency of sister chromatid exchange is extremely high (Schubert *et al*., 1980) [72].

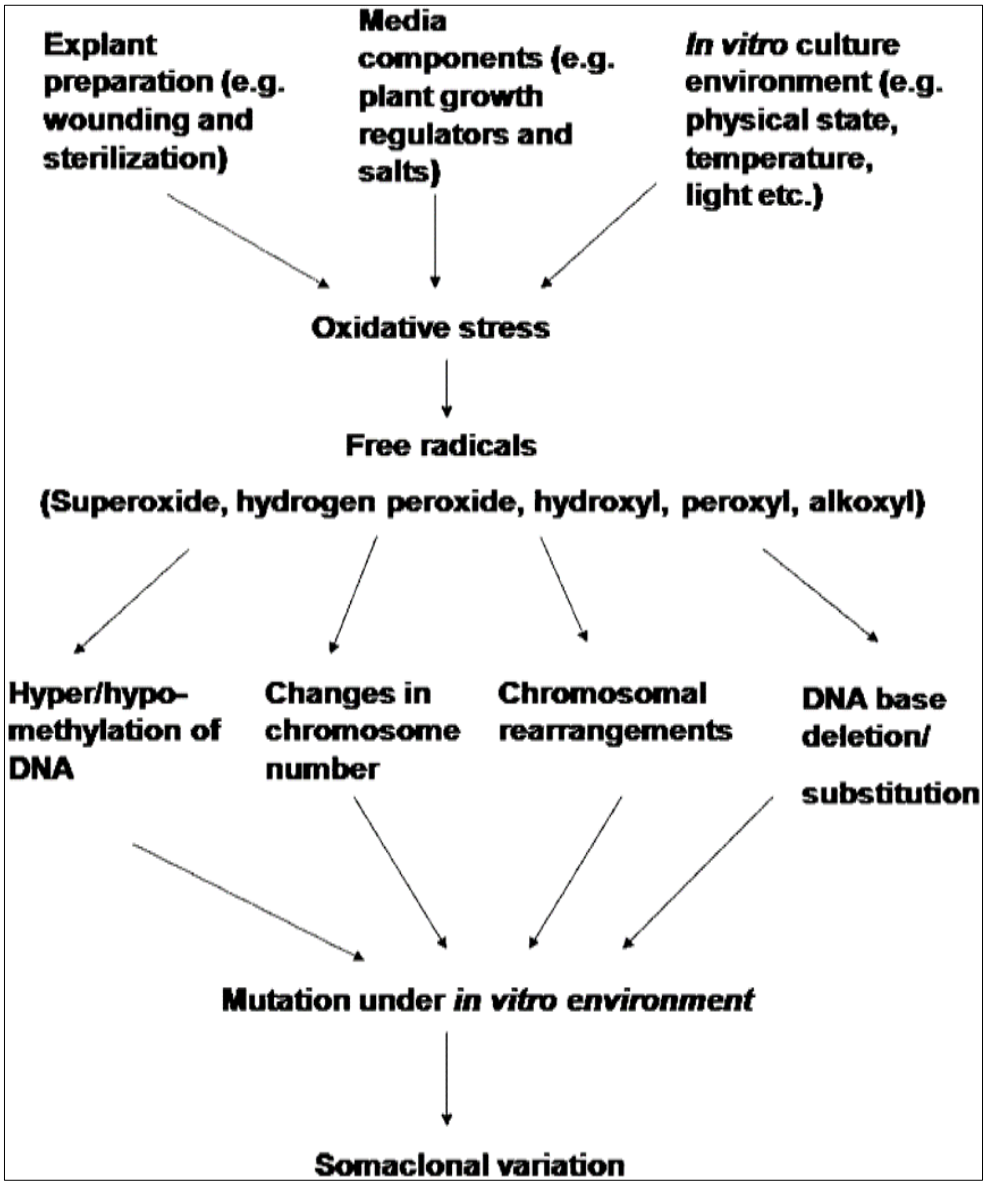

Fig 1: Mechanism of Somaclonal variation in micropropagated plants as a result of *in vitro* oxidative burst.

Source Factors Influencing Somaclonal Variations

Explant/explant source effect

When compared to explants with pre-existing meristems, such as axillary buds and shoot tips, the amount of variation produced by tissues with a high diversity, such as roots, leaves, and stems, is greater (Duncan, 1997) [14].

Table 1: The occurrence of Somaclonal variation as influenced by the explant selection.

S No.	Crop Species	Explant/ Source of Explant	Presence or absence of Somaclonal Variation (+/-)	Refrences
1	Almond (*Prunus dulcis*)	Axillary branching	-	Martins *et al*. (2004) [50]
2	Chimeric 'Marciconog' banana	Vegetative and floral Axis tip	+	Krikorian *et al*. (1993) [36]
3	Cavendish group of bananas (*Musa spp.*) Banana cv. Martaman	Chimeric shoot tip Shoot tip	+ -	Israeli *et al*. (1995) [31] Ray *et al*. (2006) [67]
4	Kiwifruit (*Actinidia deliciosa* Chev. Liang and Ferguson) cv. 'Tomuri'	Leaf blades and petioles	+	Prado *et al*. (2007) [66]
5	Papaya (*Carica papaya* L.)	Axillary shoot tips underwent cryopreservation.	+	Kaity *et al*. (2009) [33]
6	Sweet cherry (*Prunus avium*)	Shoot apical portions	+	Piagnani and Chiozzotto (2010) [65]
7	Rootstock Mr.S 2/5, selected from a half-sib progeny from *Prunus cerasifera* Erhr	Leaf	+	Muleo *et al*. (2006) [53]
8	*Vitis* Spp.	Nodal segment	-	Alizadeh *et al*. (2008) [1]

DNA Methylation

Some crops in micro propagation, prefers somatic embryogenesis as a pathway for propagules bulking. In beginning of Deoxyribonucleic acid's development, somatic embryogenesis shows lower level of methylation as compared to the later stages (Munksgaard *et al*., 1995) [54]. The reason behind the variation in the methylation status of DNA may be due to a) enzymes catalyzing methylation get activated or/and formation of *de novo*, or b) catalyzing enzyme of demethylation reaction gets inhibited or decreased., or c) alterations in the co-factors and substances taking part in these reactions (Munksgaard *et al*., 1995) [54]. It is reported, that in somatic cell during DNA hypomethylation a state of differentiation is induced which resembles to that of early zygotic embryos (Okkels, 1988; Herman, 1991) [58, 25]. In the beginning of embryogenesis there is a drop in levels of methylation in the initial followed by an increase in the later stages of embryo (Lo Schiavo *et al*., 1989) [45]. When any gene's methylation occurs, it turns off the transcription of that gene, by that it controls the gene expression during somatic embryogenesis (Duncan, 1997) [14].

Effect of culture age/number of subculture cycles

The longer the plants are grown in the culture, the greater the variety of their offspring. Because of the *in vitro* environment and the quick growth, the tissue's genetic stability may be compromised, which could lead to somaclonal variations. (Martinez *et al*., 1998) [49]. This can be ascribed to both a) the accumulation of mutation rate over time and b) an increase in mutation rate per cell generation over time (Duncan, 1997) [14]. As a result, as the frequency of variation grows, so does the number of multiplication cycles.

Effect of Plant Growth Regulators and Hormonal Factors *In vitro*

Chromosome number abnormalities were also caused by a high concentration of a mixture of BA and adenine (Zhenxun and Hongxian, 1997) [93]. The somaclonal variation frequency in platelets generated by somatic embryogenesis was either lower than or equivalent to that which was frequently found with shoot-tip propagated plants. (Shchukin *et al*., 1997) [73].

Adaptability of genotype

The basic goal of a micropropagation programme is to create clonal planting material that is true to type. Representational Difference Analysis (RDA) has recently been used to detect culture-induced variation and distinguish DNA variations between normal plants and culture-induced off type. Generally, *in-vitro* conditions for plant cells may be quite stressful, callus maintenance/induction, triggering highly mutagenic processes during explant establishment, embryo development, and regeneration of plants (Lorz *et al*., 1988) [46]. To monitor genomic integrity changes, a PCR-based approach is used in which fragments of RDA (Representational Difference Analysis) DNA have been sequenced and primers that may be used in it have been created (Thomas *et al*., 2002) [83]. According to Thomas *et al*. (2002) [83], the data support the idea that there is a particularly labile portion of the genome that is highly vulnerable to stress, with higher rates of rearrangement and mutation than the rest of the genome. As an outcome, it is clear that somaclonal variation is not an event that is random, as commonly assumed, because specific loci show higher mutation rates as compared to other during *in vitro* cultivation (Xie *et al*., 1995; Thomas *et al*., 2002) [91, 83].

The impact of ploidy, karyotype changes, and post-transcriptional processes.

Plants regenerated *in vitro* show greater variance across polyploid and high chromosomal number explant donor species than among low ploidy and low chromosomal number explant donor species. This is because polyploids and high chromosomal number explant donors have more copies of their genomes (Watson *et al*., 1992, Skirvin *et al*., 1994) [87, 76]. Breaks in chromosomes can also directly generate mutations, either through the positional effect or by changes in gene expression brought on by rearrangements of the broken up chromosomes (Peschke and Phillips, 1992) [62]. Plant cell proliferation *in vitro* from a disordered callus stage may be followed by changes in genotype or phenotype, resulting in regenerated plants that are different from the original clone. These differences can be seen in the regenerated plants' morphology, size, and coloration (Lindsey and Jones, 1989) [43].

Chromosomal breakage or aberrant chromosomal numbers can also be seen in plants that are typically grown from suckers; however, the incidence of these occurrences is far higher in plants that have been formed through tissue culture.

It is essential for there to be genetic stability among somaclonal variants if the selected features are valued and the variations are stable (Hwang and Tang, 2000). Non-segregating/homozygous variations do not arise at random, and micropropagation may promote certain mutations affecting specific DNA sequences (Xie *et al*., 1995) [91]. The tissue specific methylation of DNA get influenced when there is a transition from a linear to stationary growth phase during culture age, genotype, plant growth and primary cultures. Because numerous genes may be impacted at the same time, DNA methylation may enhance quantitative trait variance (Phillips *et al*., 1990; Munksgaard *et al*., 1995) [64, 54].

Role of transposable element

The activation of transposable elements can result in the mutation of a single gene, which may or may not be stable, as well as changes in the methylation of DNA and the activation of further transposable elements, as well as more chromosome breaks (Peschke and Phillips, 1992) [63]. In plants that are created through tissue culture or their progenies, the activation of transposable elements on their own can be regarded a symptom of genetic instability (Duncan, 1997) [14]. The events like transposition can play a significant role in somaclonal variation also tissue culture environment can be very conductive DNA transposition sequence (Larkin and Scowcroft, 1981) [38].

Genomic status of donor plant

Donor plant genomic status: Cells are often maintained as diploid in plant apical meristems (root and shoot tips), where synthesis of DNA is rapidly followed by karyokinesis and cytokinesis (normal cell cycle). Meristematic cells, on the other hand, during mitosiscells do not divide normally, but instead undergo duplication of DNA and endoreduplication during differentiation. Cells that contain 4C, 8C, or even larger quantities of DNA can be found as a result of varied degrees of endoreduplication taking place within them. The process of polyploidization of somatic cells is referred to as the phenomenon of polysomaty.

Endomitosis, also known as chromosome duplication that occurs within an intact nuclear membrane, and/or endoreduplication both occur during the process of *in vivo*

tissue formation, which leads to polysomaty. (During interphase duplication of the chromatids of each chromosome but chromosome numbers remain same). Endoreduplication is frequently connected with polysomaty, and polysomaty may be a result of differentiation (Bhojwani and Razdan, 1983) [4]. Nuclear irregularities may be discovered in polysomatic species by removing cells from the organism's stable environment and placing them in the foreign environment of the culture vessel, which amplifies nuclear irregularities (Sunderland, 1977) [79]. The stability of chromosomal number in cultivated cells does not rule out cytogenetic instability. Minor and substantial alterations in chromosome structure can result in differences that do not affect chromosome number. In some circumstances, exposing cells to mutagenic therapy will not enhance the frequency of spontaneously occurring variants. The diversity found in long term cultures is the cumulative effect of variability contributed by the mother plant and that caused by cultures condition (Bhojwani and Razdan, 1983) [4].

Table 2: The strengths and weaknesses of several marker systems for assessing clonal fidelity:

Advantages	Disadvantages
Morphological chracterstics Visual Distinction There is no need for laboratory facility Appropriate for preliminary detection	Sensitive to ontogenic and other environmental alterations Numbers are limited. Time-consuming
Cytological indicators (Flow-cytometry) In the case of flow-cytometry, sample preparation and analysis are simple and quick. Rapid and effective approach for routine large-scale ploidy level investigations Unfailing detection of even the minor changes in chromosomal number	In flow -cytometry, cytosolic compounds may interfere with quantitative DNA labelling. In the event of inflow, there is a lack of a set of internationally agreed on DNA reference criteria. Chromosome counting is time-consuming.
Markers of Isozyme Expression that is codominant Performance comfort	Sensitive to ontogenic and other environmental alterations Numbers are limited. Not all of these reagent systems are effective for all plant species. Specific tissue expression
DNA Markers Expression that is codominant For the analysis, any source DNA can be used. Neutral in phenotype Not affected by ontogenic changes and other environmental variables. Capability to identify culture-induced alteration in DNA sequence and methylation pattern.	RAPD markers are dominating and do not allow heterozygous individuals to be scored. Furthermore, they only recognise sequence alterations. ISSR is a multilocus method, hence there may be non-homology of comparable sized pieces. The necessity for pure, high molecular weight DNA, the dominance of alleles, and the likely non-homology of comigrating fragments from distinct loci are all disadvantages of AFLPs. If enough primer sequences for the crop species of interest are lacking, SSR markers will incur substantial development expenses. Furthermore, mutations in the primer annealing region might result in null alleles (no amplification of the desired PCR product), which can lead to scoring errors.

Table 3: *In vitro* selection of desirable features and generation of several economically exploited variations in diverse Fruit crops using Somaclonal variation.

S. No.	Horticultural crops	Characteristics of Somaclone	Refrences
1	Apple (*Malus domestica Borkh.*)	Resistance to *Erwinia amylovora*	Chevreau *et al.* (1998) [8]
2	Apple rootstocks M 26 and MM 106 (*Malus pumila* Mill.)	Resistance to *Phytophthora cactorum*	Rosati *et al*. (1990) [68]
3	Apple rootstock Malling 7	Resistance to white root rot (*Dematophora necatrix*)	Modgil *et al.* (2012) [52]
4	Banana (*Musa acuminata* L.)	Semi-dwarf and resistant to *Fusarium wilt* TC1-229. Larger bunch size var. TC2-425; Resistant to *Fusarium oxysporum* f. sp. cubense (Foc) race 4; bunch 40% heavier than cv. Formosana. *Fusarium wilt*-resistant somaclonal variants of banana cv. asthali Var. CIEN-BTA-03, resistant to yellow Sigatoka 10 somaclones; GCTCV215-1 released for commercial planting Var. CUDBT-B1, reduced height and early flowering Var. Tai-Chiao No. 5, superior horticultural traits and resistance to *Fusarium wilt*	Tang *et al*. (2000) [81] Hwang (2002) [26] Ghag *et al*. (2014) [17] Gimenez *et al*. (2001) [18] Hwang and Ko (1992, 2004) [26, 27] Martin *et al*. (2006) [48] Lee *et al.* (2011) [39]
5	Blackberry	Thornless var. 'Lincoln Logan'	Hall *et al*. (1986) [20]
6	*Citrus spp.*	Resistant to *Phoma tracheiphila* Salinity tolerance	Deng *et al.* (1995) [10] Ben-Hayyim and Goffer (1989) [3]
7	Java citronella (*Cymbopogon winterianus*)	Somaclonal variant variety IMAP/Bio-13, which yields 37% more oil and 39% more citronellon than the control variant	Mathur (2010) [51]
9	Kiwi fruit (*Actinidia deliciosa*)	5 somaclones, derived from cv. Tamuri, tolerant to NaCl	Caboni *et al.* (2003) [6]
10	Mango (*Mangifera indica*	Resistant to *Colletotrichum gleosporiensis*	Litz *et al*. (1991) [44]

	L.)		
11	Myrobolan (*Prunus cerasifera* Erhr)	Water logging-tolerant clone variant (S.4) of myrobolan rootstcock Mr.S 2/5 for peach cv. Sun Crest	Iacona *et al*. (2013) [30]
12	Olive (*Olive europea*)	Bush olive somaclone (BOS), columnar olive somaclone (COS)	Leva *et al*. (2012) [41]
13	Peach (*Prunus persica* L.)	Somaclones S156 and S122 resistant to leaf spot, moderately resistant to canker in cvs. Sunhigh and Red haven Resistant to root-knot nematode (*Meloidogyne incognita* Kofoid and White) Somaclone S 122-1 was found resistant to bacterial canker (*Pseudomonas syringae* pv. *syringae*)	Hammerschlag and Ognjanov (1990) [22] Hashmi *et al*. (1995) [23] Hammerschlag (2000) [21]
14	Pear (*Pyrus spp.*) Pear rootstock (*Pyrus communis* L.) 'Old Home 9 Farmingdale (OHF 333)'	Resistant to *Erwinia amylovora* Tolerance to the fifire blight	Viseur (1990) [86] Nacheva *et al.* (2014) [57]
15	Pineapple (*Ananas comosus* L., err.)	Spineless variant Cvs. P3R5 and Dwarf, variation in fruit color, growth habit, fruit size and length of plant generation cycle	Jaya *et al*. (2002) Pe´rez *et al*. (2009, 2012) [60, 61]
16	Quince A (*Cydonia oblonga*)	High soil pH	Dolcet-Sanjuan *et al*. (1992) [12,], Marino *et al*. (2000) [47]
17	Strawberry (*Fragaria sp.*)	Resistant to *Fusarium oxysporum* f. sp. *fragariae* Resistant to *Alternaria alternate* Resistant to *Phytophthora cactorum* Improved horticultural traits Resistant to *Verticillium dahliae* Kleb 'Serenity', a paler skin-colored, late season, resistant to powdery mildew and *Verticillium* wilt somaclonal variant of the short-day cv. 'Florence'	Toyoda *et al*. (1991) [84] Takahashi *et al*. (1993) [80] Battistini and Rosati (1991) Biswas *et al*. (2009) [5] Zebrowska (2010) [90] Whitehouse *et al*. (2014) [89]
18	Sweet orange (*Citrus sinensis* (L.) Osb.)	Somaclone of OLL (Orie Lee Late) sweet orange; late maturing; suitable for fresh market or processing, exceptional juice quality and flavor	Grosser *et al*. (2015) [19]

Advantages of Somaclonal Variation

There are several advantages to somaclonal variation

1. It is less expensive than other approaches to genetic manipulation and does not necessitate 'containment' procedures.
2. There are more plant species available for tissue culture than can currently be controlled by somatic hybridization and transformation.
3. In the case of transformation, it is not necessary to have identified the trait's genetic origin or even isolated and cloned it.
4. Among somaclones, novel variants have been found, and evidence suggests that transit through tissue culture can alter the frequency and distribution of genetic recombination events. This means that variation can arise from locations in the genome that are not accessible to conventional and mutant breeding (Karp 1992) [34].
5. Chimeric expression cannot be obtained when somaclones are produced in cell culture (Evans 1989) [15]. Crops with restricted genetic systems (e.g., apomicts, vegetative reproducers) and/or small genetic bases have fared well in terms of somaclonal variety. For example, in the case of ornamental plants, the use of *in vitro*-generated diversity has become routine practise in many commercial breeding operations.

Somaclonal Variations Have a Disadvantage

1. One of the significant disadvantages of somaclonal variation that makes it difficult to apply is that, despite the identification of elements influencing a certain plant species' variation response, the outcome of a somaclonal programme cannot be predicted because it is random and lacks reproducibility (Karp, 1992) [34]. Furthermore, because the bulk of genetic changes are produced by point mutations or chromosome rearrangements, the R1 population segregates. As a result, it is practically hard to choose individuals with improved R1 generation for quantitative attributes such as yield. Despite the fact that many horticultural crops have established procedures for choosing somaclones resistant to various biotic and abiotic stresses, there are currently no *in vitro* selection methods for complex qualities such as yield, soluble solids, sweetness, texture, or shelf life (Evans, 1989) [15].
2. If somaclonal variation is heritable and genetically stable, plant breeding can incorporate it. Somaclonal alterations have only been exploited in a few promising variants thus far. This could be due to a breakdown in communication between plant breeders and tissue culture experts, as well as the unpredictability of somaclones (Jain, 2001) [32]. Furthermore, while somaclonal variation has resulted in new varieties, improved variants were not selected in many cases because (1) the variations were all negative; (2) positive changes were also altered in negative ways; (3) the changes were not novel; or (4) the changes were not stable after selfing or crossing (Karp 1992) [34].

Somaclonal Diversity in Horticulture Crops

It is widely accepted that somaclonal variants originating from a different tissue culture environment are widespread in clonally produced plants and that they can be employed to generate novel variation in horticulture crops (Karp, 1995) [35]. To reap the benefits of such variants, however, effective tools for detecting, analysing, identifying, and enhancing resistant clones must be created (Sahijram *et al*. 2003) [70]. Crop improvement by somaclonal variation enables breeders to develop plants that are resistant to biotic and abiotic

challenges such as drought, high salinity, high or low soil pH, and disease (Yusnita *et al*. 2005) [92].

Case Study
Somaclonal Variation in Tissue Culture
An Olive Case Study
A. R. Leva, R. Petruccelli and L.M.R. Rinaldi

Olives are members of the genus Olea (family Oleaceae). The genus has 35 species, the most important of which is O. europaea, which has two subspecies, subsp. sylvestris (wild olive) and subsp. europaea (European olive) (cultivated olive).

The following olive regeneration system was used
Micropropagation

Micropropagation, as defined by Schaeffer, is the *in vitro* clonal growth of plants from shoot tips or nodal explants.

Somatic embryogenesis
Field performance of *in vitro*-raised olive plants
Plant morphological characterization acquired from nodal explants

The benefactor plant, which was an Olea europaea cv. Maurino tree that was 20 years old, was used as the donor plant (DP) for the initial explants that were used for micropropagation. In the field evaluation, the control plant consisted of the original donor plant that had been propagated using cuttings. In the year 1998, 70 MPs that were 16 months old and 20 control plants were grown in containers. It was decided to shift the control plant (Cp) to an experimental area in San Pancrazio, which is located in Florence, Italy. (43°39'36.00" N, 11°11'25.80" E).

During the growing phase in the field, there were no discernible differences between the MP and Cp plants in terms of the primary vegetative characteristics. According to the data, there were no differences in the growth habit, vegetative growth, canopy, or trunk area of the tree. Only the leaves and drupes of MP plants were slightly wider than those of Cp plants, but they maintained the distinctive leaf and drupe morphologies that are characteristic of the cultivar. The production of fruiting stems was comparable despite the fact that there was a little different number of flowers per inflorescence; since olive flowers only have a 2–5 percent chance of setting fruit, the slight variance had no impact on the amount of fruit that was produced. Both the MP and the CP plants possessed identical pit characteristics.

Vegetative characters	Inflorescence and fruit characters
HP Plant height: measured in meter from the soil level to the highest point	*IL Inflorescence length in mm
CP Canopy projection to the soil: measured at the two widest diameters in m2	NF Number of flowers per inflorescence

The following characteristics were observed

Vegetative characters	Inflorescence and fruit characters
VP Canopy volume in m^3	NO Number of olive fruits per fruiting shoot
TA Trunk area in cm^2	FL Fruit length in mm
VSG Vegetative shoot growth in cm	FW Fruit width in mm
VSN Node number of vegetative shoots	FL/W Fruit length/width
VSI Internode length of vegetative shoots in cm	FFW Fruit fresh weight in g
*FS Number of feather shoots (lateral shoots developing from axillary buds formed in same year) on the vegetative shoots	FDW Fruit dry weight in g
*FG Feather shoot growth in cm	PL Pit length in mm
*FN Feather shoot node number	PW Pit width in mm
*FI Internode length of feather shoots in cm	PL/W Pit length/width
LBL Leaf blade length in mm	PFW Pit weight in g
LBW Leaf blade width in mm	FFW/PW Fruit weight/Pit weight
BL/W Blade length/width	FY Production weight in Kg
LA Leaf area in mm^2	
*LFW Leaf Fresh weight in mg	
*LDW Leaf Dry weight in mg	
*DW Dry weight mg per 100 mm^2	

Molecular characterization and genetic fidelity assessment of plants generated via micropropagation:

At the end of the second year of field culture, a first molecular analysis was carried out on a total of five plants, including MP, Cp, and donor specimens. Twenty-one different primers with a 10-base sequence were used for the PCR-RAPD analysis. The primers resulted in the production of 182 different amplification fragments in total. There were no differences in the amplification pattern between the MPs, the Cps, or the donor plant, since all of the primers produced the same type of monomorphic amplification pattern in the MPs.

In 2006, RAPD and ISSR analysis were performed on 12 randomly selected 9-year-old mature MPs. Using the DNeasy Plant Mini Kit, total genomic DNA was extracted from fresh leaves of the MPs and donor plant (Qiagen, Hilden, Germany). The RAPD and ISSR data were compared among all MPs, as well as between MP and Cp plants, to detect any genetic alterations. The 40 RAPD primers yielded 301 scoreable band classes, while the 10 ISSR primers yielded 46 replicable fragments. The RAPD primer amplification products in MPs and donor plant were monomorphic, while the ISSR primers produced monomorphic bands within MPs and between MP samples and donor plant. The genetic stability and homogeneity of Olea europaea cv. Maurino MPs were validated by molecular analyses conducted in two distinct experiments at different plant ages and utilizing two types of molecular markers. Furthermore, the trustworthiness of the morphological analysis results was established.

Morphological analysis of plants regenerated through somatic embryogenesis

In the year 1992, fifty somatic seedlings were recovered from embryogenic long-term cultures that had been ongoing for three years. These seedlings were then planted in small commercial pots of 1.5 litres each and raised in a greenhouse. Following a period of three months, the survival rate was determined to be 83%. Growing somatic seedlings and MPs from the Frangivento donor plant, which acted as a putative control (Pc), in large pots inside of a greenhouse was the method of choice up to the year 1997.

During the process of growing pot, the somatic plants exhibited developmental behaviour that was distinct from that of the MPs in terms of their pace of growth and their general pattern. San Donato, which is located in Florence, Italy at 43°33'46.08" North and 11°10'21.00" East, was the location in 1998 where 43 somatic plants and 10 PC plants were transferred to field conditions. The somatic plants maintained the distinct behavioural patterns of development seen in the containers even when grown in the outdoors.

The plants were monitored over a number of years, during which time morphological variation could be identified in relation to potential yield, inflorescences, fruits, and the characteristics associated with them. There were a total of 32 morphological characteristics that were investigated. In the field, we collected data for two variant phenotype groups known as bush olive somaclones (BOS; four representative trees) and columnar olive somaclones. These observations were based on preliminary observations of plant growth in pots. (COS; four representative trees).

Molecular analysis of Somaclonal olive plants

The 20 RAPD primers produced 198 scoreable band classes with sizes ranging from 2200 to 210 bp; the number of primer bands ranged from seven (CD11 and OPA01) to thirteen (OPP10, AH30 and OPP12). Both somaclone types and Frangivento shared a substantial proportion (86%) of RAPD markers, implying that the somaclones and Frangivento share homology.

Molecular analysis of Somaclonal olive plants

Primer	Fragments (pb)	COS	BOS	DP
OPP10	1698	–	–	+
	603	–	+	–
	425	+	–	–
AH29	1190	–	+	–
	1135	+	–	+
	800	+	–	+
AG1	550	–	+	–
	260	+	–	+
OPP12	1170	–	+	+
	1350	–	+	–
	832	–	–	+
	692	–	–	+
OPA07	1114	+	–	+
	1072	+	–	+
	517	+	–	+
	407	–	+	–
	340	–	+	–
OPP14	1230	–	+	+
	725	+	+	–
	625	+	–	+
	210	+	–	+
OPP02	1260	+	–	+
	800	+	–	–
	750	–	+	–
	530	+	–	+

Primer	Fragments (pb)	COS	BOS	DP
OPP15	1000	–	+	+
	537	+	–	–
	375	–	+	+

Description of morphological variation among the vegetative progeny of somaclonal plants

Type of habitus	Rooting (%)	Root number	Root length (cm)
Columnar (COS)	64.41 n.s	5.5 n.s	2.45 n.s
Dwarf (BOS)	61.15 n.s	4.8 n.s	2.57 n.s

Type of habitus	Plant Height m	Trunk diameter cm	Length of lateral shoots cm	Node number of lateral shoots	axillary shoot number	Node number of axillary shoots
Columnar (COS)	3.2a	11.1ns	76.1a	39.5a	1.9c	5.9b
Dwarf (BOS)	2.4b	10.0ns	65.9b	31.8b	11.8a	11.6a
Putative control	3.0a	9.4ns	55.8c	27.6b	6.0b	5.7b

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