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Research Application Summary

Implications of *Somaclonal* variation *in vitro* in crop management: A review

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Abstract

Soma clonal variation presents an enormous opportunity in the horticultural industry for crop improvement in regards to using stable, heritable variations. The goal of micro-propagation of maintaining genetic infidelity and integrity resulting in clonal regenerants. In contrast to the latter, most often, in vitro conditions and, procedures do significantly account for epigenetic and genetic variability which leads to a mutation called soma clonal variation (SV). This ultimately may avail novel genotypes change that is invaluable if the variabilities are stable and heritable. Additionally, variability inducer (DNA methylation, transposons, karyotype change, mitotic crossing over, gene amplification polyploidy, aneuploidy) can lead to change(s) in gene expression or the genetic DNA sequence. In vitro variations can be isolated either by using selection or selection pressure against abiotic and biotic traits resistance. Some variations could increase undeniably the genetic diversity for plant species with narrow genetic bases, enhance useful secondary metabolites production in plants, evolutionary base phenotypic plasticity of plants response to environmental stresses through increased resistance. Consequently, most of these variations are unpredictable, less stable, non-heritable and not useful (like sterility) to their progeny. The epigenetic variations are most common and it requires intensive field trials to ascertain their suitability, stability and heritability. Embracing and utilizing the occurrence of useful soma clonal variation will contribute to knowledge bridge and crop improvement in line with resistance to pests, diseases and drought in the horticultural sector. This can significantly ameliorate food insecurity in many agricultural-dependent communities accredited to crop improvement to adapt to the current environmental condition.

Keywords: Crop improvement, epigenetic and genetic, *in vitro*, Soma clonal variation

Résumé

La variation soma-clonale présente une énorme opportunité dans l'industrie horticole pour l'amélioration des cultures concernant l'utilisation de variations stables et héréditaires. L'objectif de la micro-propagation est de maintenir l'infidélité et l'intégrité génétiques pour obtenir des régénérants clonaux. Contrairement à cette dernière, le plus souvent, les conditions et les procédures in vitro ne tiennent pas compte de manière significative de la variabilité épigénétique et génétique qui conduit à une mutation appelée variation clonale somatique (SV). En fin de compte, cela peut donner lieu à de nouveaux génotypes, ce qui est d'une importance inestimable

si les variabilités sont stables et héritables. En outre, les inducteurs de variabilité (méthylation de l'ADN, transposons, changement de caryotype, croisement mitotique, amplification génique, polyploïdie, aneuploïdie) peuvent entraîner des modifications de l'expression génétique ou de la séquence génétique de l'ADN. Les variations in vitro peuvent être isolées en utilisant la sélection ou la pression de sélection contre la résistance aux caractères abiotiques et biotiques. Certaines variations pourraient augmenter indéniablement la diversité génétique pour les espèces végétales à base génétique étroite, améliorer la production de métabolites secondaires utiles dans les plantes, fonder sur l'évolution la plasticité phénotypique de la réponse des plantes aux stress environnementaux par une résistance accrue. Par conséquent, la plupart de ces variations sont imprévisibles, moins stables, non héritables et non utiles (comme la stérilité) par leur progéniture. Les variations épigénétiques sont les plus courantes et nécessitent des essais intensifs sur le terrain pour vérifier leur pertinence, leur stabilité et leur héritabilité. La prise en compte et l'utilisation des variations soma-clonales utiles contribuera à atténuer les gaps de connaissances et à l'amélioration des cultures en fonction de la résistance aux parasites, aux maladies et à la sécheresse dans le secteur horticole. Cela peut diminuer de manière significative l'insécurité alimentaire dans de nombreuses communautés dépendantes de l'agriculture, accréditées pour l'amélioration des cultures afin de s'adapter aux conditions environnementales actuelles.

Mots-clés: amélioration végétale des cultures, épigénétique et génétique, in vitro, variation soma-clonale

Introduction

In vitro culture presents an enormous opportunity often due to the occurrence of various forms of variations (Espinosa-Leal *et al*., 2018; Bednarek and Orłowska, 2020). A useful variation could avail a comprehensive solution strategy to the prevailing stress challenges to plants presented by nature (Bairu *et al*., 2011; Leva *et al*., 2012; Leva and Rinaldi, 2017). Su *et al*. (2021) reaffirmed the novel concept of totipotency by Haberlandt (1902) based on an unequivocally illustrative experiment by Schleiden (1838) and Schwann (1839) on cell theory justify plant cell plasticity. The continuous plasticity response adaptation displayed by plants is due to recurrent unpredictable and dynamic environment changes. There is an increased occurrence of different forms of variation induced by plants' environmental responses causing somaclonal variation (SV) in vitro (Sun *et al*., 2013; Leva and Rinaldi, 2017; Martin *et al*., 2018). Additionally, in vitro culture has been rendered as the most powerful at transforming plant genes (Sun *et al*., 2013). This directly enhances molecular breeding programs vital in crop improvement in line with the current climatic conditions.

The term soma clonal variation was coined and has been defined according to several pieces of literature (Larkin and Scowcroft, 1981; Kaeppler *et al*., 2000; Bairu *et al*., 2011; Radchuk *et al*., 2012; Krishna *et al*., 2016; Martínez-Estrada *et al*., 2017; Martínez, 2018) as a variation exhibited by plants regenerated in vitro causing either genetic or epigenetic variation among plant progeny. This could be a result of pre-existing traits in the explant or induced by a tissue culture process of plant regeneration and dedifferentiation in vitro which lead to a phenotypic expression as a result of either genetic or epigenetic variabilities (Sun *et al*., 2013; Karki, 2020; Mulwa, 2021; Rebouças *et al*., 2021). According to Bairu *et al*. (2011) and Germanà *et al*. (2020) who cited Braun's illustrative experiment (1959), which noted the first soma clonal variation observation

and reported in vitro.

Subsequently, the recurrent occurrence of genetic instability during in vitro culture process of many crops species have been linked to causing a unique phenomenon 'soma clonal variation' as described by Larkin and Scowcroft (1981); Tawar *et al*. (2016); Martínez-Estrada *et al*. (2017); Espinosa-Leal *et al*. (2018); Manchanda et al. (2018); . Most plant tissue culture techniques' goals are; i) clonal (true-to-type) regenerants, ii) maximize genetic fidelity, integrity, and stability of the regenerate as the stock material (Kokina *et al*., 2017). Contrary to this expectation, an undesirable phenomenon particularly soma clonal variation is a common occurrence in the plant in vitro culture process (Kumar and Reddy, 2011; Krishna *et al*., 2016; Martínez-Estrada *et al*., 2017; Singh and Singh, 2021). Consequently, in vitro culture conditions in most cases acts as a source and/ or grounded notch for variability leading to some useful variations particularly soma clonal variation (Kar *et al*., 2014). The surge in the quest for mass production of clean planting material among most framers has accelerated the need for tissue cultured planting materials.

However, Leva *et al*. (2012)'s report elucidate illustratively how soma clonal variation genetic change unravel natural variabilities which can be useful to plant breeders particularly to increase genetic diversity for the plant with a narrow genetic base and ground base for crop improvement success. These have been accredited to the soma clonal variation incidence and plants derived as a result of soma clonal variation are called soma clones (Leva and Rinaldi, 2017).

Materials and methods

This review was carried out to document some prime tools used in crop improvement. The study topic employed a critical and systematic approach by using searched terms such as sources of soma clonal variations induced in vitro; effects of in vitro condition to explant; use of in vitro variation in crop improvement; variation stability and their heritability; to review the existing variation inducer and their implications to crop improvement. More search terms additionally such as isolation; limitation of soma clonal variations was employed. These were subsequently applied in the searching process in scientific bodies such as Research4life, Web of Science, Google Scholar, Scientific journals (e.g., Taylor and Francis, Elsevier, Springer). Some gray literature related to the subject matter; common use of in vitro variation, strategies to utilizes in vitro variation and use of various web pages to warrant a pool of varied literature. This was used to address the criticism associated with the use of peer-reviewed literature that may rely on a certain level of the subjectivity of the practitioner knowledge in the valuation of the various source of information. It also avoids the effects caused by the inconsistencies from the grey literature that may weaken the value of the work thus so, was used together with or backed up by empirical literature.

The basis of soma clonal variation *in vitro.* Soma clonal variation is a phenotypic variation either genetic or epigenetic in origin (Bahadur *et al*., 2015). Genetic (permanent) or epigenetic (reversible and temporary) variations are the most common among in vitro-regenerated plants (Singh, 2015; Mulwa, 2021). The genetic and phenotypic variability mostly arise due to epigenetic effects or changes in gene expression such as a change in the chromosome number which have been induced by in vitro tissue culture procedures or pre-existing in the stock plant (Noormohammadi *et al*., 2020; Samantara *et al*., 2021). Alternatively, soma clonal variation can be categorized either somatically or meiotically steady occurrence or events that, result in mutation. However, not all

soma clonal variations are stable, heritable meiotically and particularly reversible epigenetic variation (Wang and Wang, 2012; Nwauzoma and Jaja, 2013).

Several illustrative pieces of literature (Bhatia, 2015; Germanà *et al*., 2020; Karki, 2020; Sivakumar *et al*., 2011; Tawar *et al*., 2016) cites that permanent, stable and inherent soma clonal variation, provided noble sources of genetic variation based tool for crop improvement, resistance to disease, improved quality, or higher yield. This has been used to improve crops such as citrus, bananas, sugar cane, cowpeas, turmeric, potato, tobacco, cereals, brassica species (Bairu *et al*., 2011; Germanà *et al*., 2020; Tawar *et al*., 2016). Consequently, this variation significantly varies in range from a specific trait to the whole plant genome or virtually in a portion of the genome but unless it affects a visible or measurable trait that can enable its identification (Noormohammadi *et al*., 2020). When this generates stable plants traits load which are heritable traits, it could lead to a breakthrough for monoembryonic cultivars improvement such as clementine (Germanà *et al*., 2020). However, manifestation and chances for most genetic diversity and variability within a plant species or variety genome or population are often generated in nature due to the recombination process presumed to also leads to soma clonal variations. This is also attributed to several influential factors such as natural selection, mutation, migration and population size that induce or activate genetic variability in various ways occurring during the tissue culture process (Kar *et al*., 2014; Krishna *et al*., 2016). This leads to various types of soma clonal variation morphology, environmental tolerance, physiology and yield.

Induction of soma clonal variation. The induction of soma clonal variations recurrently arises from cultures that rely regularly on adventitious regenerations (somatic embryos or shoots and roots) and callus (Manchanda et al., 2018). Conversely, soma clonal variations are found to be caused by either pre-existing traits, tissue culturally induced, epigenetic and genetic (Manchanda *et al*., 2018). This presents the basis of induction of the variation. Some variations such as soma clonal variation mostly occur to plants regenerated in vitro particularly from callus, not with exception from other techniques. Pre-existing or induced in vitro variation leads to phenotypic or genetic alteration which originates from a mutation that is either epigenetic or genetic (Bhatia, 2015; Bhatia and Sharma, 2015).

The parent or stock plant genotype which acted as a source of the explant to start in vitro culture act as a primary source of variation (Leva and Rinaldi, 2017). This often ensures genetic fidelity for in vitro regenerated plants. Pre-existing variations are attributed to the possible presence of mutated cells present in the explant (chimeras, somatic cells, callus, cells of different ploidy levels). However, frequent variation occurrence in plant tissue cells affecting the gene expression such as chimeric nature of the genotype, effects of somatic cell, callus or cell suspensions usage is attributed as sole cause due to their ability to rapidly multiply which in turn lead to preexisting or intrinsic spontaneous mutations happening naturally in plants causing induced variability (Ghag *et al*., 2014; Leva and Rinaldi, 2017). Pre-existing variations in vitro culture are presumed as the principal cause of mutations, epigenetic changes, or a combination of both mechanisms (Bairu *et al*., 2011).

Tissue culture processes induction. *In vitro* culturing of explants involves additional components like artificial condition (light quality, varying photoperiods and temperature), nutritional media, additives such ass exogenous plant growth regulators (Leva and Rinaldi, 2017). Consequently,

prominent in vitro tissue cultured induced variations such as cell anomalies, transposal element, rearrangement of chromosomes, cell cycle regulation factors, activation of cryptic transposable elements epigenetic variation do account for the induction of soma clonal variation (Deepthi, 2018). Mode of regeneration such as callus phase, somatic embryogenesis and protoplast preparation techniques are prone to chromosome variability as it introduces varying magnitude and type of stress imposed on cultured cells during in vitro culture (Bhojwani and Dantu, 2013; Rebouças *et al*., 2021). Sub culturing length or increased numbers of cycles are presumed to induce soma clonal variation (Krishna *et al*., 2016). Alternatively, exogenous rationing of plant growth regulators such as auxins and cytokinin can induce callus from the somatic cell which is friable to changes leading to variation.

Epigenetic variation. Epigenetic variations (developmental variations) are in vitro physiological changes ascribed to cell structural adaptation of chromosomes that are either temporary, non-heritable and reversible phenotypic changes in response to environmental conditions (Bhatia and Sharma, 2015; Manchanda *et al*., 2018; Noormohammadi *et al*., 2020). These are changes that are capable of causing mutation not due to change in the genetic but rather change in gene expression(Bhatia, 2015). Changes resulting from epigenetic variation are linked to increasing plant resistance through storage formation memories against most environmental stresses (Ramos-Cruz *et al*., 2021). Occurrence is often during callus induction, growth and regeneration within the in vitro procedures (Wei *et al*., 2016). Chromatin structures are prone to epigenetic mechanisms change, alter the gene expression such as increase or decrease in chromosomal leading to cytological changes (ploidy levels change, aneuploidy, polyploidy) which ultimately leads to morphological and phenotypic variation (Wang and Wang, 2012; Singh, 2015; Wei *et al*., 2016; Samantara *et al*., 2021). Additionally, pre-embryogenic stages of the culture process such as callus initiation and maintenance facilitate the formation of soma clones for example male fertility, transient dwarfism and partial fertility and occurrence of thorns in juvenile Citrus (Bhatia, 2015; Manchanda *et al*., 2018).

Genetic variation. The irreversible change in the genome of the plant during the tissue culture process leading to permanent, stable and hereditable traits that are likely to persist in the progeny of regenerants plants (Bairu *et al*., 2011; Noormohammadi *et al*., 2020). This can be due to mutation such as DNA methylation (point mutation, chromosome breakage, insertion, deletion and substitutions) (Ghosh *et al*., 2021), DNA amplification (histone methylation, histone deacetylation, phosphorylation, and carbonylation). Karyotype change, cytogenetic abnormalities, gene activation or inactivation, insertions of transposable elements and retrotransposons are often common among in vitro regenerated plants as a principal source of variabilities (Wang and Wang, 2012; Bednarek and Orłowska, 2020). Subsequently, chromosome breakage occurs during the normal cell cycle which prevents cell division before completion of DNA replication are prone to disruption, deletions, duplications, transitions, transversions and activation of various transposable processes through excision and insertion present a higher possibility for soma clonal variation occurrence (Manchanda *et al*., 2018). Morphogenic changes such as structural rearrangements and extensive chromosomal loss due to nucleotide pool imbalance and heterochromatin replication are liable to variability(Manchanda *et al*., 2018; Pawełkowicz *et al*., 2021). Genetic variabilities are stable and heritable but rare, once realize can be useful in crop improvement. There are many causes of soma clonal variation in vitro.

Causes of *in vitro* variation. *In vitro* process of propagation, the use of the adventitious mean of propagation and occurrence of callus culture increases the chances for variability, more so noted under extreme stress that leads to upsurge frequent levels of mutation that can result in developmental and heritable variations (Manchanda *et al*., 2018; Rebouças *et al*., 2021). Indirect somatic embryogenesis and organogenic differentiation as well do increase the chances and occurrence level of DNA methylation and callus formation (dedifferentiation) thus further the chance of chromosomal variability. Somatic differentiation (cryptic chromosomal changes, polyteny, endopolyploidy and amplification of DNA sequences) and leads to variation (Ghag *et al*., 2014).

Genotype and explant source. The origin and genetic makeup of explant particularly from somatic cells, method of regeneration and the source of regenerants are the prime cause of variation in vitro (Sahijram, 2015). Explants from leaves, roots, internodes, ovaries, from callus cell, has higher chance to cause variability which later causes soma clonal variation (Ghosh *et al*., 2021). The duration of cell culture; This has a direct or indirect effect as increasing the number and duration enhances the rate of soma clonal variations particularly in cell suspension and callus cultures. There is a positive correlation between an increase in DNA methylation rate and the addition of auxins into the culture medium of cell suspension cultures (Manchanda *et al*., 2018). The length of storage of the culture can lead to chimeric happening. Limiting the number of subculture cycles to about 5- 8 cycles is moderately less prone to mutation as a result of variability caused by the in vitro processing and its conditions. For example, doubling the duration of culture in tobacco protoplast increased its genetic variability by 6% (Sahijram, 2015).

In vitro stress conditions; Oxidative stress if induced may lead to the formation of reactive oxygen species (Bednarek and Orłowska, 2020). This often involves the activates DNA methylation (hyper and hypo), deletion and substitution leading to a change in the chromosomal number and structure (Rebouças *et al*., 2021). DNA amplification, segregation of chimeras' tissue from pre-existing in the explant positively contributed to soma clonal variation in the plant (Manchanda *et al*., 2018, p. 311). Heritable 'gene inactivation' such as putative mutation and homozygous mutations for example a jointless-pedicel mutant in tomato, a yellow-seeded mutant in Brassica juncea and two dwarf plants in rice are a recent form of soma clonal variation in vitro culture (Manchanda *et al*., 2018).

Plant growth regulators concentrations; Different exogenous plant growth hormones have a positive correlation to the formation of soma clonal variation in vitro for example auxin is mostly used for induction of callus and cell cultures, in turn, friable to variability (such as polyploidy) leading to variation. Also, the plant growth regulators such as 2, 4-dichloro phenoxy acetic acid (2, 4-D) and naphthalene acetic acid (NAA) in the culture medium increases the chance for the formation of callus which is prone to variability such as karyotypic alterations (Bhojwani and Dantu, 2013; Pawełkowicz *et al*., 2021). This led to mutation causing a variation in the regenerant plant. Additionally, variations can be isolated basically in two ways.

Isolation of soma clonal variation. Isolation involves the process of screening and cell selection (Deepthi, 2018). Screening involves physical observation of a large number of cells or regenerated plants for detecting variants and visible variation such as yield traits like cell clones which produce a high amount of certain bio-chemicals (Manchanda *et al*., 2018). This involves long-term treatment

but in a stepwise manner as a culture is indiscriminately exposed to a selective concentration such as polyethylene glycol or mannitol (screening of drought-tolerant trait) in a gradual manner. Cell selection depends entirely on the selection pressure imposed to allows survival or growth of desired variants only dubbed as positive selection. At the cellular level by growing cells from cell suspensions and callus culture and subjected to the higher concentration level of nutrient supplements such as antibiotics and chemicals to induce stresses (biotic and abiotic), those that survive are regenerated (Bhojwani and Dantu, 2013; Manchanda *et al*., 2018). This is important for specific selection such as cells resistant to toxins, herbicides, high salt concentration. There are different methods used to detect the occurrence of this variation in tissue culture.

Method used to detect soma clonal variation. Phenotypic or morphological markers are used based on the identification of phenotypic markers such as quantitative characteristics for example variances and irregularities in plant seeds, pigmentation, stature, leaves, plant height and leaf and fruit morphology (Bairu *et al*., 2011; Manchanda *et al*., 2018). This is more feasible for already established in the field or greenhouse not with in vitro culture since the regenerant is not well established (Leva and Rinaldi, 2017).

The use of cytological and physical/biochemical markers which are capable of identifying and noticing numerical, structural, chromosomal alterations and ploidy change causing variation by analysis procedure by chromosome counting and flow cytometry (Krishna *et al*., 2016; Leva and Rinaldi, 2017; Manchanda *et al*., 2018). Such markers detect variability in proteins and isozymes and are more effective in identifying soma clonal variation. However, it is time-consuming and sensitive to the environment.

Molecular markers molecular tools, random amplified polymorphic DNA (RAPD) analyses, DNA-based alteration, inter-simple sequence repeat (ISSR), simple sequence repeat, and amplified fragment length polymorphism (AFLP) have been used in detecting in vitro variation. Therefore, the combination of these methods increases efficiency and accuracy level in detecting somaclonal variation in vitro (Leva and Rinaldi, 2017; Manchanda *et al*., 2018). However, AFLP has proven as the most effective and powerful PCR-based marker system frequently employed tool in the detection of soma clonal variation (Bairu *et al*., 2011). Useful variations in vitro have been embraced as a potential tool in many agricultural sectors regarding crops improvement.

Application of useful soma clonal variation. There is an increase in the identification of useful variation in vitro basically for crop improvement. Tissue culture has often generated an array of epigenetic variations that can be incorporated into plant breeding programs so as develop and improved more diverse environmentally adapted varieties or cultivars without the use of external genes (transgenic plants) and present a chance for in vitro selection (Sahijram, 2015; Wei *et al*., 2016; Ghosh *et al*., 2021).

Soma clonal variation has been used in the development and production of various resistance traits such as disease resistance (rice, wheat, apple, tomato), abiotic stress resistance (aluminum tolerance in carrot), salt tolerance (tobacco and maize), herbicide resistance (tobacco resistant to sulfonylurea), and plants with improved quality of seeds (low content of neurotoxin seeds of Lathyrus sativus), resistance to nematodes and atrazine eggplant (*S. melongena*), callus from leaves of tomato have developed into varieties which allow mechanical harvesting (Manchanda

et al., 2018). They may also be useful for mass propagation of improved individuals without lowering the risks of returning to the wild type. However, the rise in genome reshuffling and rearrangement in vitro culturing presents a chance for alien gene insertion into the crop and it widens the crop germplasm base (Bahadur *et al*., 2015; Rebouças *et al*., 2021). In most conditions, DNA methylation can be inherited by subsequent generations due to its stability for example in some plant species such as Myrtus communis and almonds (Bhatia and Sharma, 2015; Sahijram, 2015).

Some in vitro variations are more useful for increased the production of secondary metabolites invaluable plants such as herbal used in the horticultural and pharmaceutical industries (Nwauzoma and Jaja, 2013; Deepthi, 2018). The stable variation can be stored and preserved by cryopreservation. This very useful for endangered species as enhance environmental conservation (Bairu and Kane, 2011; Leva *et al*., 2012; Bhojwani and Dantu, 2013). However, in vitro variation are faced with some limitations which impede their use in crop improvement.

Limitation of soma clonal variation. Generally, most soma clonal variations may not be useful for example sterility. Often a common incidence arising from somatic embryogenesis and micro-propagation processes (Leva and Rinaldi, 2017). Not appropriate for complex agronomic traits like yield, quality as most and novel variants are not transferable through conventional methods. This variation does occur in an uncontrolled and unpredictable manner and many cell lines (calli) may not exhibit regeneration possibilities (Sahijram, 2015; Leva and Rinaldi, 2017). Soma clonal variations are frequently unstable, non-heritable, random and epigenetic in nature. Therefore, their occurrence depends on the regeneration potential of pre-existing traits cultivars and may have poor plant regeneration without new variation (Bairu *et al*., 2011). This requires extensive field trials to assure the suitability and stability of soma clonal variants and the expression of the pleiotropic effect. Furthermore, soma clonal variation is comparatively difficult to predict, the techniques and equipment for its identification are limited and expensive (Bahadur *et al*., 2015; Krishna *et al*., 2016).

Conclusion

Soma clonal variation is caused by tissue culture conditions leading to epigenetic and genetic variability thus lead to mutation. As a result of the valuable contribution of soma clonal variation in the horticultural sectors such as the development of variation in photosynthetic, agronomical desirable traits, and resistance and or tolerance to biotic and abiotic stresses in response to the diverse, dynamic versatile environment to revolutionize newly sustainable and adapted varieties. In this regard, such development and adapted varieties are significant to the context of current environmental challenges experienced globally. Owing to pleiotropic effects, there is a need for depth understanding, quest to predict their occurrence in tissues culture to indoctrinate more information and knowledge on their use and forge various ways to activate stable, useful, and heritable variations.

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