

Research Application Summary

Somaclonal variation in plant tissue culture: A review

Otieno, C., Nyalala, S. & Ogwenno, J.

Crops, Horticulture and Soils Department, Faculty of Agriculture, Egerton University,
Nakuru 536-20115, Kenya

Corresponding Author: constance.otieno@yahoo.com

Abstract

Somaclonal variation is a form of variation which occur in plants or tissues obtained from tissue culture, giving rise to unique genotypes in horticulture. This variation is majorly brought about by mutations linked to various stress factors, including and not limited to wounding, residues of sterilants used during sterilization, incomplete tissues, and imbalances of media components, among others. This review aimed at outlining the basis and sources of somaclonal variation in tissue culture. Search engines including dimensions and google scholar, scientific journals from Wiley online and Elsevier, among others, were used to obtain information on somatic variation in tissue culture. The basis of somaclonal variation were found to be genetic and include changes in chromosome number, point mutations, Karyotype changes, among other factors. Somaclonal variation can be used to produce resistant plant varieties and unique horticultural plants among other uses. Even though somaclonal variation is a threat to the genetic stability of tissue culture plants, they also provide tools for improvement to plant breeders, especially for crops with a narrow genetic base.

Key words: DNA, mutations, somaclonal variation, tissue culture, variants

Résumé

La variation somaclonale est une forme de variation qui se produit chez les plantes ou les tissus obtenus à partir de la culture de tissus, donnant ainsi naissance à des génotypes uniques en horticulture. Cette variation est principalement causée par des mutations liées à divers facteurs de stress, comprenant, mais sans s'y limiter, les traumatismes, les résidus de produits stérilisants utilisés lors de la stérilisation, les tissus incomplets et les déséquilibres des composants du milieu de culture, entre autres. Cette revue vise à présenter les bases et les sources de la variation somaclonale en culture de tissus. Des moteurs de recherche tels que Dimensions et Google Scholar, des revues scientifiques en ligne comme Wiley et Elsevier, entre autres, ont été utilisés pour obtenir des informations sur la variation somatique en culture de tissus. Les bases de la variation somaclonale ont été identifiées comme étant génétiques et comprennent des changements du nombre de chromosomes, des mutations ponctuelles, des changements du caryotype, entre autres facteurs. La variation somaclonale peut être utilisée pour produire des variétés végétales résistantes et des plantes horticoles uniques, entre autres utilisations. Bien que la variation somaclonale constitue une menace pour la stabilité génétique des plantes en culture de tissus, elle offre également des outils d'amélioration aux sélectionneurs de plantes, notamment pour les cultures présentant une base génétique étroite.

Mots-clés : ADN, mutations, variation somaclonale, culture de tissus, variants

Introduction

Somaclonal variation is a form of variation occurring in plants or tissues regenerated in vitro such as callus and suspension culture (Modgil *et al.*, 2012). The implication of somaclonal variation among in vitro plants in the economy is significant in fruit crops and woody plants, because of their long life cycles. Hence, the incidence of somaclonal variation in plant tissue culture is of great interest in micro propagation systems basically because genetic assortment and variability within a population are brought about through reassimilation events.

The growth of plant cells in vitro and their reformation into whole plants is an asexual process involving only mitotic division of the cells (Ravindra *et al.*, 2012). Thus, the incidence of uncontrolled and random unpremeditated variation during in vitro plant tissue culture is a major problem (Hoque and Morshad, 2014). In vitro, the conditions of culture can bring about mutations causing rejuvenated plants obtained from organ cultures, calli, protoplasts and somatic embryos to exhibit physical and genetic variation (Kar *et al.*, 2014), leading to some or all of the somaclones exhibiting physical characteristics that differ from the mother plants (Grosser *et al.*, 2015). Somaclonal variation provides an important source of genetic differentiation for the improvement of crops through the selection of unique variants, which may show resistance to disease, improved quality, or higher yield (Mondal *et al.*, 2013). The aim of the review was to outline the basis and sources of somaclonal variation in tissue culture.

Methodology

Search engines such as dimensions, research4life, google scholar and scientific journals from Elsevier, Wiley online and springer were used to obtain information on somaclonal variations in plant tissue culture using key terms as somaclonal variation, the basis of somaclonal variation, induction of somaclonal variation and the factors affecting somaclonal variation in plant tissue culture.

Results

Basis of somaclonal variation. A number of suggested basis for somaclonal variation include changes in chromosome number (Bridgen *et al.*, 2018), point mutations, Karyotype changes, mitotic or somatic crossing over and sister chromatid exchange, (Yamin and Hama, 2017), somatic gene rearrangement, DNA amplification and methylation (Tiwari *et al.*, 2013), changes in organelle DNA or chromosome structure (Al-Khayri and Naik, 2011), chromosome breakage and rearrangement (Bendif *et al.*, 2017), histone modifications and RNA interference (Miguel and Marum, 2011) and segregation of preexisting chimeral tissue (Ravindra *et al.*, 2012).

Induction of somaclonal variation in tissue culture. Somaclonal variations in tissue culture are mainly caused by newly initiated mutations emerging from tissue culture process. The activators of mutations in tissue culture has been linked to several stress factors, whereby the variations exhibited in micropropagated plants could be resultant of or relative to oxidative stress damage imposed upon plant tissues cultured in vitro (Nivas and Dsouza, 2014). This leads to increased amounts of reactive oxygen species (ROS), which are linked to alteration of DNA chromosome number from polyploidy to aneuploidy, chromosome strand breakage, chromosome rearrangements, and DNA

base deletions and substitutions (Padhi *et al.*, 2018).

Factors affecting somaclonal variation in tissue culture. There are copious factors that steer somaclonal variation in plants grown in vitro leading to differential disparity in such plants. These include the explant source, the mode of regeneration, the length of culture period and number of subculture cycles, the culture environment and the genotype and ploidy.

In relation to the explant source, divergent sources of tissues used for regeneration of plants in vitro lead to contrasts in both the frequency and the nature of variation (Shiji *et al.*, 2015). Highly differentiated tissues such as roots, leaves, and stems produce more variations than explants with preexisting meristems, such as axillary buds and shoot tips (Krishna *et al.*, 2016).

The mode of regeneration is another factor affecting somaclonal variation in tissue culture. This includes culture induction and subsequent subcultures which expose explants to oxidative stress resulting in mutations (Sarmah *et al.*, 2017). The severity depends on the tissue culture technique, whereby cultures obtained from callus stimulate increased mutation rate as opposed to plants obtained via axillary branching (Manchanda *et al.*, 2018). This is attributed to the different category of disturbance, as in the first case, the cells follow a normal division pattern observed in a developing plant while for callus, a dedifferentiation phase occurs followed by uncontrolled cell divisions (Nwauzoma and Jaja, 2013).

The length of culture period and the number of subculture cycles also lead to somaclonal variation in tissue culture. The longer a culture is sustained in vitro, the greater the somaclonal variation (Sun *et al.*, 2013). Variant karyotypes are found to escalate with increasing age of callus and consequently, the chances of variant plants produced during continuous subculture also increases (Nassar *et al.*, 2014). Khan *et al.* (2014) reported that after the eighth subculture, the number of somaclonal variants increased with a concomitant decrease in the reproduction rate of propagules in banana. Similarly, Clarindo *et al.* (2012) suggested a maximum of less than four months storage of coffee cell aggregate suspensions for true-to-type mass propagation as ploidy vulnerability was noticed in long-term in vitro culture. The time scale of subcultures also leads to increased rate of somaclonal variations, especially among cell suspension and callus cultures (Sun *et al.*, 2013). Studies have shown that somaclonal variation is more detectable in plants regenerated from long-term cultures. Rival *et al.* (2013) noticed that in vitro proliferation generates DNA hyper methylation overtime leading to regulation of the expression of embryogenic capacity of oil palm during tissue culture.

External environmental factors pertaining to the culture environment like growth regulators, temperature, light, osmolarity and agitation rate of the culture medium are known to effect the cell cycle in vivo in plants, indicating that insufficient control of cell cycle in vitro is one of the origin of somaclonal variation (Nwauzoma and Jaja, 2013).

The genotype and ploidy play a crucial role in in vitro ontogeny as some differences are genotype specific (Eftekhari *et al.*, 2012). Among factors affecting somaclonal variation, plant genotype is the principal determinant of variation (Nwauzoma and Jaja, 2013). Leva *et al.* (2012) characterized oil palm clones as low/moderate risk and high risk with regard to 'mantle' flowering, on the basis of terminal inflorescence data generated under in vitro conditions. Clones grouped as high risk at

the outset gave a higher incidence of mantled flowering in the field than low/medium risk clones, confirming that data on terminal inflorescences produced in vitro allows effective screening of material with regard to the risk of mantled flowering.

Application of somaclonal variation in important horticultural crops. Somaclonal variations in in vitro cultured plants bring about new variants in potato, strawberry, tulip, banana, chilli and pepper. Qualitative traits such as flower color, plant height, fruit shape, fruit color, flowering habit are improved in selected variants (Nhut *et al.*, 2013). Varieties resistant and tolerant to biotic stress developed from in vitro genetic changes are applied in rough lemon and Kiwi fruit (Rajan and Singh, 2021). Banana – ‘Tai-Chiao No. 5’ and ‘S.4’ in myrobalan, are the variants selected having resistance to Fusarium wilt and tolerance to water logging respectively (Anil *et al.*, 2018).

Conclusion

Commercial feasibility of micro propagation technology depends on maintenance of genetic precision in the regenerated plants. Despite extensive studies conducted on somaclonal variation, the means in which it occurs still remains unknown. However, variation in most cases is linked to the molecular foundation of the plant which is related to DNA methylation. Therefore, thorough genetic information from mother crops is pivotal for somatic variation to be applicable to a wide variety of crops, and to establish the genetic precision of in vitro regenerated plants. Thus, the morphological and cytological assays should be key for the sustained success of fidelity tests related to the production of clonal plants.

Acknowledgement

This paper is a contribution to the 18th RUFORUM Annual General Meeting and Scientific Conference held 12-16 December 2022 in Harare, Zimbabwe.

References

- Al-Khayri, J. and Naik, P.M. 2017. Date palm micropropagation: Advances and applications. *Ciência e Agrotecnologia* 41(4): 347-358.
- Bendif, H., Mohammed, L., Noura, G.M.M. and Mohamed-Amine, K.F.K. 2017. Exploitation of somaclonal variability for the search of saline-tolerant potato (*Solanum tuberosum* L.). *International Multidisciplinary Research Journal* 08-12.
- Bridgen, M.P, Van Houtven, W. and Eeckhaut, T. 2018. Plant tissue culture techniques for breeding. pp. 27-144. In: ornamental crops. Springer, Cham
- Clarindo, W.R., Carvalho, C.R. and Mendonça, M.A.C. 2012. Ploidy instability in long-term in vitro cultures of *Coffea arabica* L. monitored by flow cytometry. *Journal of Plant Growth Regulators* 68(3): 533–538.
- Eftekhari, M., Alizadeh, M., Mashayekhi, K. and Asghari, H.R. 2012. In vitro propagation of four Iranian grape varieties: influence of genotype and pretreatment with *arbuscular mycorrhiza*. *Journal of Vitis* 51:175–182.
- Grosser, J.W., Gmitter, J.F.G., Dutt, M., Calovic, M., Ling, P. and Castle, B. 2015. Highlights of the University of Florida, citrus research and education center’s comprehensive citrus breeding and genetics program. *Acta Horticulturae* 1065: 405-413.

- Hoque, M.E. and Morshad, M.N. 2014. Somaclonal variation in potato (*Solanum tuberosum* L.) using chemical mutagens. *The Agriculturists* 12 (1):15-25.
- Kar, B., Kuanar, A., Singh, S., Mohanty, S., Joshi, R.K., Subudhi E. and Nayak, S. 2014. In-vitro induction, screening and detection of high essential oil yielding somaclones in turmeric (*Curcuma longa* L.). *Plant Growth Regulation* 72(1): 59-66.
- Khan, S.A., Rahman, L.U., Shanker, K. and Singh, M. 2014. Agrobacterium tumefaciens-mediated transgenic plant and somaclone production through direct and indirect regeneration from leaves in *Stevia rebaudiana* with their glycoside profile. *Journal of Protoplasma* 251(3): 661–670.
- Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M. and Sadh, R.K. 2016) Somaclonal variations and their applications in horticultural crops improvement. *Journal of Biotechnology* 6 (1): 1-18.
- Leva, A.R., Petruccelli, R. and Rinaldi, L.M.R. 2012. Somaclonal variation in tissue culture: A case study with olive. *Recent Advances in Plant in-vitro Culture* 7:123-150.
- Manchanda, P., Kaur, A. and Gosal, S.S. 2018. Somaclonal variation for sugarcane improvement. In *Biotechnologies of Crop Improvement*. Springer, Cham., 1: 299-326.
- Miguel, C. and Marum, L. 2011. An epigenetic view of plant cells cultured in vitro somaclonal variation and beyond. *Journal of Exp Bot* 62:3713–3725.
- Modgil, M., Guleria, N., Ghani, M. and Sharma, J.N. 2012. Identifying somaclonal variants of the apple rootstock Malling 7 resistant to white root rot. *Journal of Scientia Horticulturae* 137: 148-155.
- Mondal, T.K., Bantawa, P., Sarkar, B., Ghosh, P. and Chand, P.K. 2013. Cellular differentiation, regeneration, and secondary metabolite production in medicinal *Picrorhiza* spp. *Plant Cell, Tissue and Organ Culture (PCTOC)* 112 (2): 143-158.
- Nassar, A.M., Kubow, S., Leclerc, Y.N. and Donnelly, D. J. 2014. Somatic mining for phytonutrient improvement of ‘Russet Burbank’ potato. *American Journal of Potato and Respiration* 91:89–100.
- Nivas, S.K. and Dsouza, L. 2014. Genetic fidelity in micropropagated plantlets of *Anacardium occidentale* L. (Cashew) an important fruit tree. *International Journal of Science and Respiration* 3: 2142–2146.
- Padhi, M., Singh, S., Singh, P. and Mishra, N. 2018. In-Vitro Regeneration of lasora (*Cordia myxa* Roxb.) USING xb.) using nodal segments. *The Bioscan Journal* 13 (2): 541-544.
- Rajan, R.P. and Singh, G. 2021. A review on application of somaclonal variation in important horticulture crops. *Journal of Plant Cell Biotechnology and Molecular Biology* 22 (35-36):161-175.
- Ravindra, N.S., Ramesh, S.I., Gupta, M.K., Jhang, T., Shukla, A.K., Darokar, M.P. and Kulkarni, R.N. 2012. Evaluation of somaclonal variation for genetic improvement of patchouli (*Pogostemon patchouli*), an exclusively vegetatively propagated aromatic plant. *Journal of Crop Science and Biotechnology* 15(1): 33-39.
- Rival, A., Ilbert, P., Labeyrie, A., Torres, E., Doulebeau, S., Personne, A., Dussert, S., Beule, T., Durand-Gasselín, T., Tregear, J. and Jaligot, E. 2013. Variations in genomic DNA methylation during the longterm in vitro proliferation of oil palm embryogenic suspension cultures. *Journal of Plant Cell Rep* 32:359–368.
- Sarmah, D., Sutradhar, M. and Singh, B.K. 2017. Somaclonal variation and its’ application in ornamentals plants. *International Journal of Pure and Applied Bio Science* 5: 396-406.
- Shiji, R., George, J., Sunitha, S. and Muthuraj, R. 2015. Micropropagation for rapid multiplication

- of planting material in cassava (*Manihot esculenta* Crantz). *Journal of Root Crops* 40 (1):23-30.
- Sun, S., Zhong, J., Li, S. and Wang, X. 2013. Tissue culture-induced somaclonal variation of decreased pollen viability in torenia (*Torenia fournieri* Lind.). *Journal of Botany Studies* 54 (1):1-7.
- Tiwari, J.K, Chandel, P., Gupta, S., Gopal, J., Singh, B.P. and Bhardwaj, V. 2013. Analysis of genetic stability of in vitro propagated potato microtubers using DNA markers. *Journal of Physiology and Molecular Biology Plants* 19:587–595.
- Yamin, M. and Hama, S. 2017. Application methods for Conventional and modern development of onion (*Allium cepa* L.) in abiotic stresses. In Proceeding International Conference on Natural and Social Science (ICONSS).