

Mini-review: Current Applications of *In Vitro*-Induced Somaclonal Variation for Plant Genetic Improvement, with Emphasis on Advances in Pineapple

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Abstract

Plant genetic improvement integrates conventional breeding with advanced biotechnological approaches to enhance traits such as yield, disease resistance, and stress tolerance. Among these, *in vitro*-induced somaclonal variation—genetic and epigenetic alterations arising during tissue culture—has emerged as a valuable tool for crop improvement. This variation can lead to novel phenotypes suitable for selection and propagation. Recent studies have demonstrated its utility in crops such as sugarcane, rice, banana, potato, wheat, tomato, barley, chrysanthemum, soybean, and maize. This review distinguishes itself by providing the first integrated evaluation of somaclonal variation applications across major crops alongside a detailed case study of pineapple, a species seldom emphasised in prior reviews. As one of the most widely cultivated tropical fruits with significant commercial value in both fresh and processed markets, pineapple plays a vital role in the agricultural economies of many developing countries. We highlight results from somaclonal variants derived from the Red Spanish cultivar, including P3R5 and Dwarf, which exhibited significant morphological and physiological differences. Amplified Fragment Length Polymorphism confirmed genetic divergence, with Dwarf showing enhanced water-use efficiency and antioxidant activity. These findings underscore somaclonal variation's potential as a complementary strategy to conventional breeding, contributing to crop diversification and agricultural resilience.

Keywords: genetic variation; *in vitro* culture; pineapple; plant biotechnology; plant genetic improvement

Introduction

1. Enhancing plant genetic improvement: methods and breakthroughs for sustainable crop development:

Plant genetic improvement refers to enhancing the genetic makeup of plants to develop superior agronomic traits. This process combines traditional methods, such as selective breeding, with modern biotechnological tools, including genetic engineering and marker-assisted selection (1-3). This approach aims to achieve desirable traits such as increased yields, enhanced resistance to pests and diseases, improved nutritional content, and higher tolerance to environmental stresses (4-6). As a cornerstone of modern agriculture, plant genetic improvement plays a critical role in ensuring global food security by addressing the challenges posed by a growing population and climate change. Recent advances in CRISPR-based gene editing have highlighted its promise for improving food and nutritional security under climate-stressed conditions, particularly in vegetable crops (6), while policy-focused frameworks have emphasised the importance of aligning biotechnological innovation with agricultural sustainability goals (7). Through crop-specific strategies and the application of tailored technologies, this approach supports the development of more resilient, sustainable, and productive cultivars for the future (8, 9). An overview of the key techniques employed in plant genetic improvement is outlined below (10-14):

1. **Selection:** This involves the identification and propagation of plants with desirable traits, performed naturally or artificially, to enhance specific characteristics such as

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yield, stress tolerance, or disease resistance.

2. Hybridisation: The crossing of two genetically distinct plants to combine their favourable traits, involving either intraspecific (within the same species) or interspecific (between different species) hybridisation.
3. Mutation genetic improvement: Using physical (e.g., radiation) or chemical mutagens to induce genetic mutations, followed by the selection of individuals with beneficial traits. This approach has been instrumental in developing novel crop varieties with improved characteristics.
4. Polyploidy induction: Artificially doubling or altering the chromosome number in plants to enhance characteristics such as size, yield, and vigour or stress resistance.
5. Genetic engineering: Inserting specific genes into a plant's genome to introduce desired traits, such as pest resistance or improved nutritional content. Examples include genetically modified crops like *Bt* cotton and golden rice.
6. Marker-assisted selection: Using molecular markers linked to desirable genetic traits, to speed up and enhance the precision of selection in breeding programmes
7. CRISPR-Cas9 and genome editing: Precision tools for targeted modification of specific genes to introduce or alter traits in plants, enabling rapid and accurate genetic improvement.
8. Tissue culture and micropropagation: *In vitro* culture of plant cells or tissues in a controlled environment to produce large numbers of genetically uniform plants, while preserving desirable traits.

Moreover, somaclonal variation refers to the genetic and epigenetic changes occurring in plants cultured *in vitro*, such as in tissue culture (15-17). These variations can arise due to factors like chromosomal rearrangements, mutations, or changes in DNA methylation patterns during the cell culture process (18-20).

Somaclonal variation presents both challenges and opportunities in plant genetic improvement. On the one hand, it can introduce unwanted variability and undesirable traits, making it a concern for clonal propagation where genetic uniformity is essential. On the other hand, when correctly handled, somaclonal variation can be harnessed for plant genetic improvement, as it is a source of valuable novel genetic diversity. For example, it has been used to develop new plant varieties with enhanced resistance to diseases, improved stress tolerance, or better agronomic performance (19, 21, 22).

2. Advantages of somaclonal variation (15, 17, 21, 23):

1. Increased genetic diversity: Somaclonal variation generates novel genetic variants that can be useful in plant breeding, providing new traits for crop improvement.
2. Disease and pest resistance: Somaclonal variation can result in genotypes with enhanced resistance to specific diseases or pests, which are highly valuable for crop protection in agriculture.
3. Abiotic stress tolerance: Plants with improved tolerance to

environmental stresses like drought, salinity, or extreme temperatures can be developed through somaclonal variation.

4. Faster improvement: By generating useful variation directly through tissue culture, somaclonal variation bypasses the lengthy traditional breeding processes, potentially speeding up the development of improved crop varieties.
5. Cost-effective improvement: Generating useful traits through somaclonal variation provides a relatively low-cost alternative to advanced genetic engineering techniques.

3. Disadvantages of somaclonal variation (15-17):

1. Unpredictability: The random nature of genetic and epigenetic changes in somaclonal variation makes it difficult to control or predict the outcome, limiting its reliability for targeted trait development.
2. Unwanted traits: Along with beneficial traits, somaclonal variation may result in undesirable phenotypes that could negatively affect plant performance, reducing its value for commercial applications.
3. Lack of stability: Some traits induced by somaclonal variation may exhibit poor heritability or instability over successive generations, limiting their long-term usefulness.
4. Time-consuming screening: Identifying plants with desirable traits in a large population of somaclonal variants can be labour-intensive and time-consuming.
5. Limited applicability: Not all plant species respond well to *in vitro* culture, restricting the use of somaclonal variation for certain crops.

4. Ten plant species that have been recently genetically improved using somaclonal variation:

1. Sugarcane - Enhanced disease resistance and improved yield (24-26).
2. Rice - Development of salt-tolerant and high-yielding varieties (27-29).
3. Banana - Improved resistance to fungal diseases like Fusarium wilt (30-32).
4. Potato - Enhanced resistance to late blight and improved tuber quality (33-35).
5. Wheat - Development of drought-tolerant and disease-resistant strains (36-38).
6. Tomato - Improved plant morphology, fruit quality and resistance to pests (39-41).
7. Barley - Enhanced tolerance to salinity and improved grain quality (42-44).
8. Chrysanthemum - Development of new ornamental traits and disease resistance (45-47).
9. Soybean - Improved resistance to pathogens (48-50).
10. Maize - Enhanced resistance to pests and improved nutritional quality (51-53).

These examples demonstrate the versatility of somaclonal variation in addressing agricultural and horticultural challenges.

5. Exploring the role of somaclonal variation in advancing pineapple genetic improvement (54-56):

The study began with the *in vitro* culture of 50 field-derived buds from *Ananas comosus* cv. Red Spanish, yielding 43 shoots after 42 days. These were micropropagated over 168 days using Murashige and Skoog (MS) medium supplemented with 9.3 μM benzyladenine and 1.6 μM naphthalene-acetic acid, resulting in a total of 24,768 shoots. From these, 300 young leaves were randomly selected to induce callus using a medium containing 29.0 μM naphthalene-acetic acid and 9.7 μM kinetin. After four months of proliferation, 500 calli (3 mm $\varnothing$) were transferred to a regeneration medium composed of MS salts, 0.9 μM indole-3-butyric acid, 1.1 μM benzyladenine, and 0.09 μM gibberellic acid, leading to 427 regenerated plantlets (54). Of the regenerated plantlets, 387 were hardened *ex vitro* and transferred to the field for two consecutive generations (30 months) of asexual propagation. Forty-four phenotypic traits – including those related to morphology, plant architecture, reproductive organs, and biochemical composition – were measured and statistically compared using ANOVA followed by Tukey's test ($P < 0.05$). The P3R5 and Dwarf somaclonal variants were identified based on significant deviations from the donor cultivar (Figure 1a).

Genomic DNA was extracted from young leaves, and Amplified Fragment Length Polymorphism (AFLP) profiling was conducted using three EcoRI/MseI primer combinations (e.g., Eco-AAT/Mse-AAG), producing 141 clear markers. Approximately 5% of these markers showed polymorphisms distinguishing both variants from the donor plant. UPGMA dendrograms based on NTSYS-pc software revealed greater genetic divergence in the Dwarf variant compared to P3R5, confirming phenotypic differentiation at the molecular level (54).

P3R5 differed from the donor in slip and sucker count and the presence of spines on leaves and fruit crowns. The Dwarf variant showed significant variations in plant height, peduncle diameter, shoots, slips, suckers, fruit size and shape, fruit colouration, leaf colour, plant architecture, and generation cycle length (Figure 1b). AFLP analysis confirmed genetic differences, with distinct banding patterns for both variants, separated by less than 0.09 units of genetic distance from the donor (54). Detailed evaluations included 44 agricultural, morphological, and physiological indicators. P3R5 differed in 19 variables (43.18%), while Dwarf exhibited differences in 31 variables (70.45%). Key distinctions included water use efficiency, chlorophyll content, transpiration rates, fruit mass, leaf parenchyma thickness, and antioxidant activity. While P3R5 and the

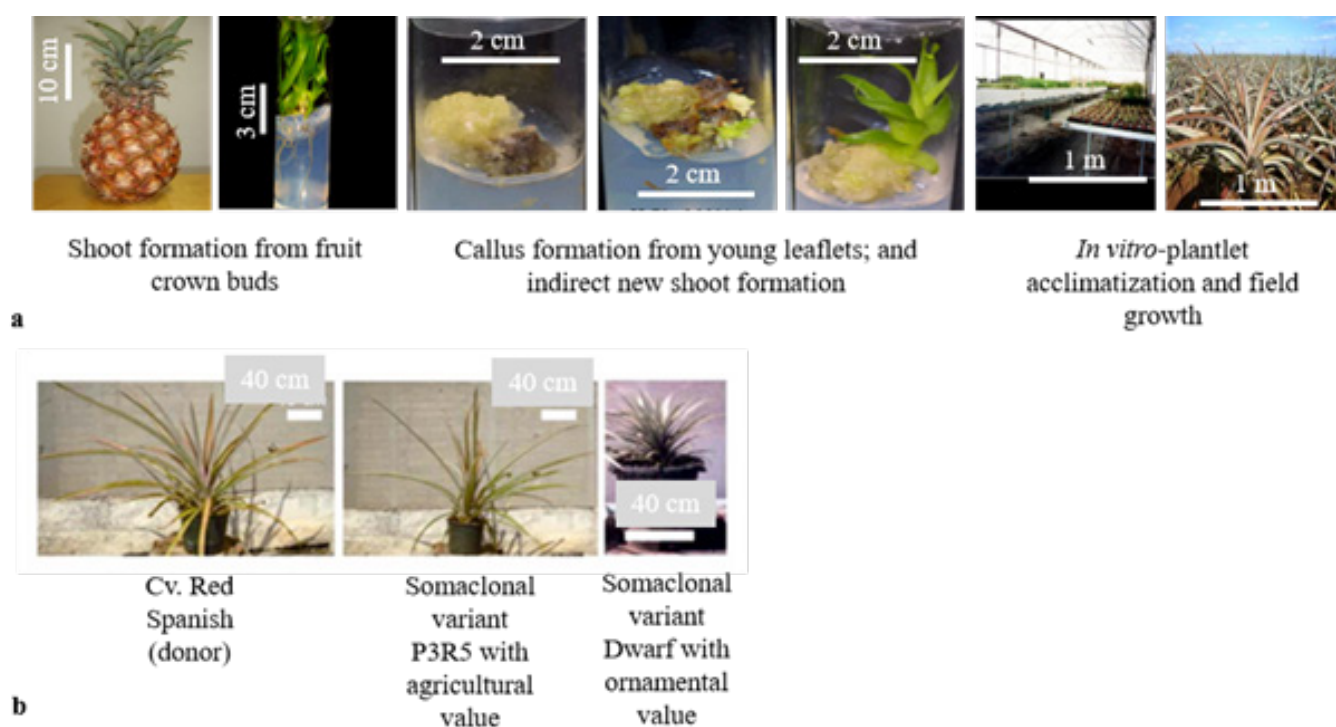


Figure 1. Workflow and phenotypic outcomes of somaclonal variation in pineapple (*Ananas comosus* cv. Red Spanish): From *in vitro* regeneration to field evaluation (original sources for the phenotypic data and AFLP validation in references [54-56](#)). (a) Tissue culture progression from fruit crown bud micropropagation, callus induction from young leaves, and subsequent regeneration into plantlets, followed by *ex vitro* acclimatisation and field establishment. (b) Representative phenotypes of the donor cultivar and two somaclonal variants: P3R5, exhibiting altered vegetative propagation traits with potential agricultural value, and Dwarf, displaying compact stature, modified fruit and leaf morphology, and enhanced ornamental potential. Scale bars provided for comparative reference.

donor formed no shoots, Dwarf produced two shoots per plant (54, 55).

Morphological studies revealed differences in stomatal traits, vascular tissue diameter, and photosynthetic efficiency. P3R5 showed no significant differences in stomatal diameter and photosynthetic rates compared to the donor. In contrast, Dwarf showed differences in nearly all parameters except stomatal diameter and transpiration rates (55, 56).

Euclidean distances between genotypes (AFLP) and phenotypes revealed that P3R5 was 2.83 units away from the donor genotype, while Dwarf differed by 3.00 units. Phenotype evaluations showed greater divergence: 3.74 for P3R5 and 4.71 for Dwarf. This study highlights the potential of somaclonal variation to drive significant phenotypic changes through subtle DNA modifications (56).

Pineapple is recognised as a key tropical fruit crop of significant economic importance (57-59). Conventional breeding in pineapple faces numerous challenges, including limited genetic variability, long generation times, and vegetative propagation constraints. These limitations underscore the value of biotechnological approaches like somaclonal variation as promising tools for developing novel phenotypes. The study summarised here represents the first comprehensive characterisation of pineapple somaclonal variants, emphasising distinct genetic and phenotypic differences amongst regenerants.

In conclusion, somaclonal variation, induced through *in vitro* tissue culture, has successfully introduced genetic diversity for crop improvement, across a range of species, including sugarcane, rice, and maize. Its potential in pineapple improvement is demonstrated by the generation of novel variants such as P3R5 and Dwarf, which exhibit distinct morphological and physiological traits. These findings highlight the potential of somaclonal variation as a valuable biotechnological solution for overcoming the limitations of traditional breeding and advancing agricultural innovation. Compared to other crops like sugarcane, rice, or maize, where somaclonal variation has yielded mutants with enhanced disease resistance or productivity, the pineapple variants P3R5 and Dwarf offer a rare and well-characterised example of stable phenotypic divergence in a clonally propagated tropical fruit. The two-generation field trial over 30 months reinforces the phenotypic stability of the variants, particularly Dwarf, whose traits – such as compact architecture and enhanced antioxidant capacity – could have both agronomic and ornamental value. Unlike cereals, where high-throughput selection is possible, the vegetative propagation and long growth cycle of pineapple make the recovery and maintenance of useful somaclonal mutants especially noteworthy. Future research should focus on integrating somaclonal induction pipelines with gene-editing technologies such as CRISPR/Cas9 to validate candidate regulatory loci underlying the observed traits. Additionally, transcriptomic and epigenomic profiling of the variants across developmental stages could reveal mechanisms of somaclonal plasticity, supporting the rational design of protocols for predictable variation in recalcitrant crops like pineapple.

Author contribution

GP, EY, MI, YA, MLTF, BEZB, OV and JCL designed the research and the review structure; analysed the data and wrote the paper. They have read and approved the final manuscript.

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Competing interest

Authors do not have any conflict of interests.

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Human and animal rights

This research did not involve experiments with human or animal participants.

Informed consent

Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Data availability

The datasets generated during and/or analysed during the current study will be available on reasonable request.

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