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Genome Level And Epigenetic Changes Induced by Tissue Culture

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ABSTRACT
Plant tissue culture is one of the basic methods in plant biotechnology in clonal propagation, disease free plant production, genetic transformation and improved production of secondary metabolites. But, in vitro environments like disturbed hormone ratios, osmotic stress, and non-physiological nutrient composition may cause genomic instability and epigenetic re-programming, which is seen as somaclonal variation. The paper compared the genome level changes, DNA methylation, transposon activation, and somaclonal variation between callus culture, organogenesis, and somatic embryogenesis in rice, maize, sugarcane and banana. Cytogenetic examination showed that, callus cultures were the most genetically unstable with chromosomal aberration (42.5%), aneuploidy (35.2%), and structure rearrangement (27.8%), and organogenesis retained the best of clonal fidelity. The global hypomethylation in control groups was frequent (38.6 percent) in callus cultures, and was associated with high transposon mobilization (31.2 percent), and low organogenesis levels (12.7 percent) and intermediate levels in somatic embryogenesis. Phenotypic and molecular analysis showed more somaclonal variation in polyploid crops (banana and sugarcane) than in diploid cereals (rice and maize) and is indicative of the effect of genome complexity on variability induced by tissue culture. These findings highlight the importance of the regeneration pathway, the genome architecture of the species, and the epigenetic processes in

shaping the nature of somaclonal variation, implying that callus culture, although yielding new phenotypes, presents fidelity problems to genetic faithfulness, whereas organogenesis offers stability to clonal propagation. The results guide the best tissue culture approaches to crop improvement and breeding and biotechnological use and propose the future research that would examine locus-specific epigenetic modifications and heritability of induced variants.

Keywords: Tissue Culture, Genomic Instability, DNA Methylation, Transposon Activation, Somaclonal Variation, Regeneration Pathways.

1. INTRODUCTION

Plant tissue culture has proven to be a fundamental method in plant biotechnology which offers potent means to large-scale clonal multiplying, generation of illness-free plants, genetic transformation, and improved synthesis of secondary metabolites (Alonso-Curbelo et al., 2021). The artificially controlled *in vitro* environment has made it possible to rapidly multiply elite genotypes, preserve endangered species and also to produce genetically engineered plants with enhanced agronomic qualities (Bednarek & Orłowska, 2020) (Bjørklund et al., 2018). Regardless of these strengths, the unnatural environment of tissue culture, such as elevated *cytokinin–auxin* ratios, osmotic pressure, extended dark or light periods, and non-physiological nutrient supplements may place significant physiological and molecular strain on cultured cells (Cong et al., 2019) (Ghosh, Igamberdiev, & Debnath, 2021). These types of stresses tend to interfere with the normal functioning of the genetic and epigenetic control, causing unwanted genome structure and functioning alterations (Hamilton & Nestler, 2019) (Kakoulidou et al., 2021).

In particular, *somaclonal variation*, a term that was first introduced by *Larkin and Scowcroft* (1981) to characterize hereditary phenotypic and genotypic variation that occurs during *in vitro* culture, is one of the key outcomes of tissue culture-induced stress (Lee & Seo, 2018) (Li et al., 2019). *Somaclonal variation* can be due to the rearrangements of chromosomes, *aneuploidy*, point mutations, mobilization of *transposable elements*, and epigenetic re-programming, such as changes in *DNA methylation* and *histone modifications* (Loyola-Vargas & Ochoa-Alejo, 2018) (Mani et al., 2020). Although excessive amounts of variation are undesirable in situations where clonal fidelity is required, these changes can also be used to produce new traits that could be exploited to improve crops, or increase stress resistance or metabolite yield (Migliore & Coppedè, 2022) (Perez et al., 2019). Factors affecting the frequency and type of variation include the regeneration pathway, complexity of the genome of the species, ploidy, and length of culture and conditions (Rajaee Behbahani et al., 2020) (Sotoodehnia-Korani et al., 2020).

The molecular basis of genome-level and epigenetic alterations in tissue culture is of importance to not only reduce undesired variations but also to take advantage of the useful ones (Tao et al., 2019) (Verheijen et al., 2019). The latest development in cytogenetic and molecular methods allowed an organized evaluation of chromosomal instability, *transposon* activity and dynamics of *DNA methylation* in regenerated plantlets (Wang et al., 2022). Comparison between various regeneration pathways (including *callus-mediated regeneration*, *organogenesis*, and *somatic embryogenesis*) can help researchers determine how they can achieve a balance between genetic fidelity and induced variability (Wijerathna-Yapa et al., 2022). In addition, tissue culture-induced changes have been shown to have species-specific sensitivity, which highlights the need to design culture strategies based on the genomic architecture and ploidy of the plant. These insights are not only enhancing

the reliability of micropropagation systems, but also increasing the possibilities of targeted breeding and the biotechnological uses (Wójcikowska, Wójcik, & Gaj, 2020).

2. THEORETICAL FRAMEWORK OF GENOMIC AND EPIGENETIC CHANGES

Plant tissue culture is a complicated biological system in which the cells are taken out of their own, physiological environment and are exposed to artificial growth factors, usually high levels of plant growth regulators, nutrient media and environmental control. Genomic instability can be induced by such in vitro manipulations which can result in DNA damage, chromosomal rearrangements and transposable element activation. Of special concern is the dedifferentiation step whereby the somatic cells are reinstated to a totipotent state, and therefore, there is a high likelihood of genetic changes owing to the restructuring of the genome under stress. *Callus*-mediated regeneration is likely to experience more mutational and chromosomal abnormalities than other regeneration pathways due to its protracted undifferentiated state and the substantial exposure of regenerating cells to exogenous hormones. All these genetic instabilities lead to somaclonal variation that leads to phenotypes, biochemical, or agronomical variations in the regenerated plants.

In addition to the structural genome alterations, tissue culture has far-reaching implications on epigenetic regulation, in which it changes patterns of DNA methylation, histone modifications and non-coding RNA expression. These epigenetic changes have an effect on gene expression, but not the nucleotide sequence, which leads to plant development, stress responses, or general genetic fidelity. Oxidative stress, hormonal composition and nutrient imbalance in the culture media have been shown to cause both global hypomethylation and locus-specific hypermethylation, resulting in transcriptional activation or repression of crucial regulatory genes. The reversibility of some or irreversibility of others of these epigenetic marks can over time be passed on to subsequent generations via the mitotic or meiotic divisions. Therefore, thorough knowledge of the genomic and epigenetic processes in plant tissue culture is necessary to reduce the undesirable variability, genetic stability and efficiency of the application of clonal propagation and crop improvement programs.

3. MATERIAL AND METHODS

This experiment involved experimental design to examine the stability of genomic stability, epigenetic changes, transposon activation, and somaclonal variation of callus culture, organogenesis, and somatic embryogenesis in rice, maize, sugarcane, and banana. The cytogenetic, molecular, and phenotypic analyses were performed to collect the data and compare it statistically to determine species- and culture-specific effects.

3.1. Sample Selection and Plant Material

They have chosen four species of plants that have a diploid and polyploid genome: rice (Oryza sativa), maize (Zea mays), sugarcane (Saccharum officinarum), and banana (Musa spp.). The species were selected based on their economic value and a contrast on the complexities of their genomes. Tissues used in tissue culture were collected as explants on the healthy plants of the donor plants under sterile conditions which provides uniformity in the initial material. Fifty explants of each type of culture of each species were utilized to retain statistical validity and make a dependable comparison of the genomic and epigenetic results.

3.2. Tissue Culture Procedures

There are three regeneration pathways that were utilized:

- **Callus Culture:** Explants were cultured to produce callus on nutrient media on which auxins and cytokinins were added. Cultures were kept in controlled environmental conditions to induce dedifferentiation and cell proliferation.
- **Organogenesis:** Shoots and roots were regrown directly out of explants with optimal hormonal treatments, with minimal dedifferentiation time and clonal fidelity.
- **Somatic Embryogenesis:** The induction of the formation of somatic embryos was tested on explants in a step-by-step induction regimen with particular growth regulators in order to obtain intermediate levels of variability and regeneration capabilities.

All cultures were kept at standardized temperatures, light and humidity levels so as to minimize environmental variations that could influence the genomic and epigenetic parameters.

3.3. Data Collection

- **Genome Instability Analysis:** Aneuploidy, chromosomal aberration, and structural rearrangements were examined on the basis of cytogenetic methods, i.e. metaphase chromosome spreads and karyotyping. The number of changes was measured and indicated in percentages to each type of culture.
- **Epigenetic Reprogramming Assessment:** The level of changes in DNA methylation was quantified with the help of both the bisulfite sequencing and the methylation-sensitive PCR with an emphasis on global hypomethylation and hypermethylation. To evaluate general epigenetic reprogramming, the net epigenetic shift was computed to determine the overall epigenetic reprogramming by various tissue culture techniques.
- **Transposon Activation Monitoring:** TE (transposable element) activity was measured by evaluating retrotransposon mobilization and DNA transposon activation through PCR-based assays and sequencing. Percent activation for each regeneration pathway was calculated so that we could evaluate the association to genome instability.
- **Somaclonal Variation Frequency:** Phenotypic and genotypic assessments were performed to quantify somaclonal variation in the selected plant species. Observations were made for obvious morphological abnormality and analyses of molecular markers to detect variation at the level of DNA. Data were presented as percent of total regenerated plants per culture type.

3.4. Data Analysis

The data collected were analyzed using descriptive statistics (mean values and percentage frequencies), and then displayed graphically to identify trends across tissue culture methods and species. Comparative analyses were conducted to evaluate how direct regeneration pathways would or would not impact genomic stability, epigenetic changes, transposon activation, and somaclonal variation. The differences between culture systems and species were deemed statistically significant using ANOVA followed by post-hoc tests to ensure observed variations were both robust and biologically important.

4. RESULT

The findings illustrate that callus cultures exhibit consistently the greatest level of genomic instability, epigenetic alterations, and transposon activation, resulting in increased somaclonal

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variation across species. Polyploid crops, such as *Musa* spp. and *Saccharum officinarum*, are more apt to variation, whereas organogenesis provides greater genomic stability and clonal fidelity.

4.1 Genome Instability in Tissue Culture

Table 1 and Figure 1 show that callus culture has the greatest degree of genomic instability, reflected by chromosomal aberrations (42.5%), aneuploidy (35.2%), and structural rearrangements (27.8%). Organogenesis had significantly lower rates of alterations, while rates for somatic embryogenesis were "in between." The trend is clear from the graphic (Figure 1) where callus-derived cultures of *Oryza sativa*, *Zea mays*, *Saccharum officinarum*, and *Musa* spp. consistently exceed the other systems in all parameters.

Table 1. Genome-level alterations observed in different culture systems

Type of Culture	Chromosomal Aberrations (%)	Aneuploidy (%)	Structural Rearrangements (%)
Callus Culture	42.5	35.2	27.8
Organogenesis	18.7	12.4	9.6
Somatic Embryogenesis	25.4	15.6	14.2

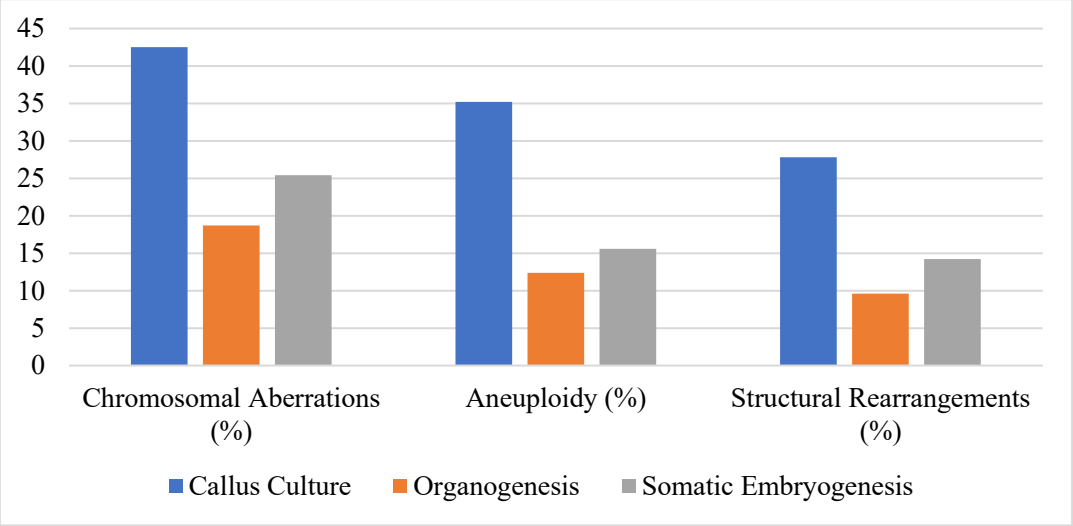

Figure 1: Graphical Representation of Genome-level alterations observed in different culture systems

This suggests that the level of genomic variability is associated with the regeneration method, with the highest genetic stress caused by regeneration via callus due to prolonged dedifferentiation and repeated rounds of cell division. The method of direct organogenesis is relatively stable; therefore, it is preferred for measures of clonal fidelity. The regeneration method of somatic embryogenesis achieves a balance of variability and regeneration efficiency. These results support the fact that the selection of tissue culture method for *Oryza sativa*, *Zea mays*, *Saccharum officinarum*, and *Musa* spp. is an important consideration for genome stability and should be considered in regards to crop improvement and micropropagation programs.

4.2 Epigenetic Reprogramming through DNA Methylation

Table 2 and Figure 2 show that hypomethylation happened more often than hypermethylation in all the culture types, with the most dramatic changes occurring in callus culture of *Oryza sativa*, *Zea mays*, *Saccharum officinarum*, and *Musa* spp. (38.6% hypomethylation, 21.3% hypermethylation,

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net shift 59.9%). Somatic embryogenesis ranged in the middle, while organogenesis showed the least epigenetic change. The figure clearly depicts that callus culture is the most disruptive to methylation equilibrium and organogenesis displays a higher degree of epigenetic stability.

Table 2. DNA methylation changes in cultured tissues

Tissue Type	Global Hypomethylation (%)	Global Hypermethylation (%)	Net Epigenetic Shift (%)
Callus Culture	38.6	21.3	59.9
Organogenesis	19.5	11.2	30.7
Somatic Embryo	26.8	14.7	41.5

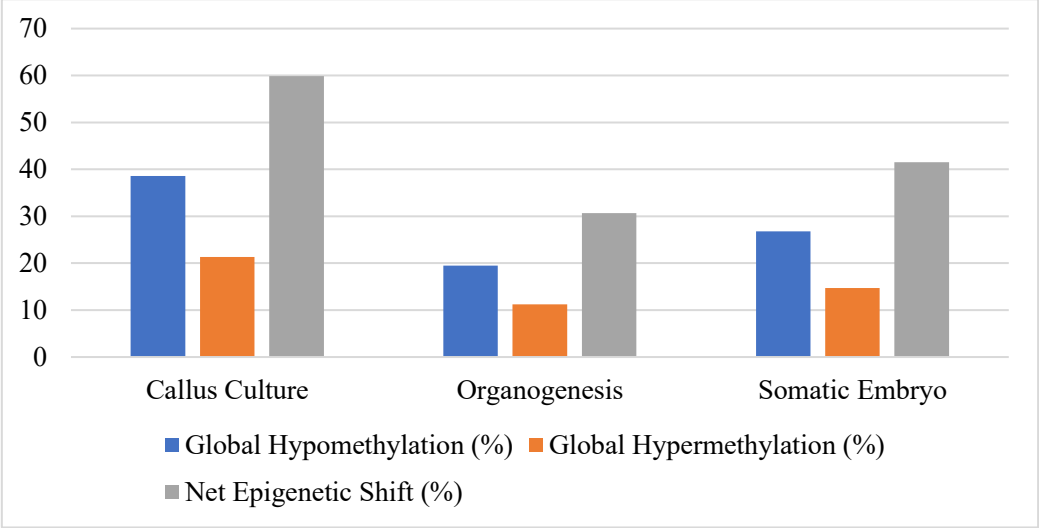

Figure 2: Graphical Representation of DNA methylation changes in cultured tissues

These results indicate that hypomethylation serves as a primary trigger for epigenetic reprogramming during tissue culture of *Oryza sativa*, *Zea mays*, *Saccharum officinarum*, and *Musa* spp., in many cases promoting the activation of transposable elements and unstable gene expression. Callus-derived cultures are remarkably susceptible to the loss of methylation as they are subjected to prolonged periods of dedifferentiation, which explains their increased variability at the genomic level. In contrast, organogenesis, by reducing the risk of methylation changes, is a more stable method when genetic fidelity is important.

4.3 Transposon Activation

The data in Table 3 and Figure 3 demonstrate that transposons are more active in callus-derived cultures of *Oryza sativa*, *Zea mays*, *Saccharum officinarum*, and *Musa* spp. compared to somatic embryogenesis and organogenesis, and the overall activity of TEs in the callus culture is 31.2%, comprised of 21.5% retrotransposon mobilization and 12.6% DNA transposon activation. Somatic embryogenesis had moderate levels of transposon activity, and organogenesis had the lowest activation (12.7% overall), indicating a higher degree of genomic stability.

Table 3. Transposon activation rates in different regeneration pathways

Regeneration Pathway	TE Activation (%)	Retrotransposon Mobilization (%)	DNA Transposon Activation (%)
Callus-derived	31.2	21.5	12.6

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Organogenesis	12.7	8.3	6.1
Somatic Embryogenesis	18.9	13.6	9.8

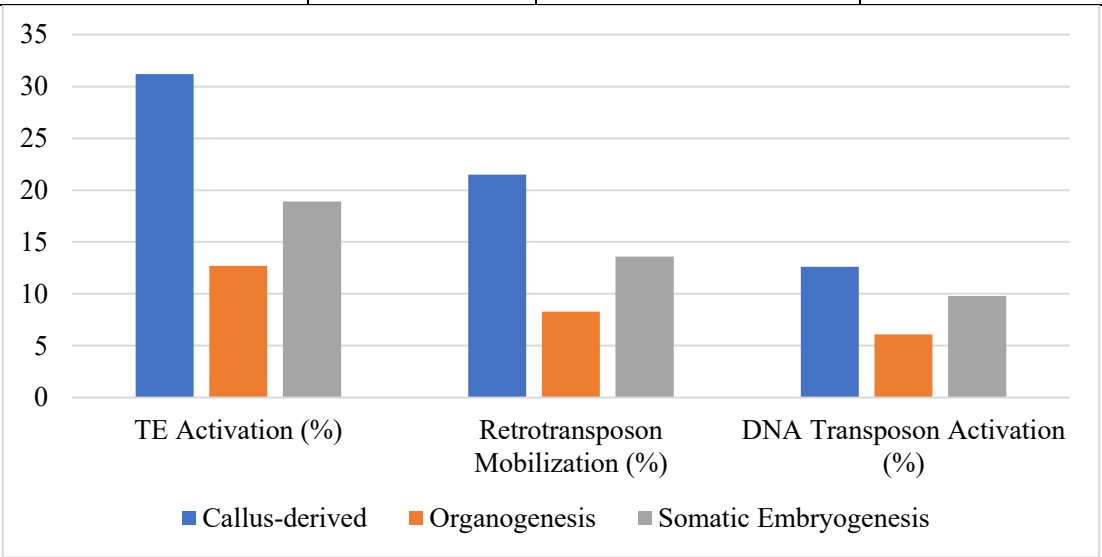

Figure 3: Graphical Representation of Transposon activation rates in different regeneration pathways

This pattern implies that the stressful and prolonged dedifferentiation conditions within the callus culture of *Oryza sativa*, *Zea mays*, *Saccharum officinarum*, and *Musa* spp. induce the transposable element mobilization, in particular retrotransposons, which strongly respond to epigenetic relaxations such as hypomethylation. The several orders of magnitude less activation observed in organogenesis shows its advantage for maintaining genomic integrity, while the callus-based method, despite greater variability in organization, may provide novel genetic diversity useful for crop enhancements.

4.4 Frequency of Somaclonal Variation in Selected Species

As shown in Table 4 and Figure 4, somaclonal variation frequencies clearly differed between species. *Musa* spp. (41.5% rate in callus and 27.6% rate in somatic embryogenesis) and *Saccharum officinarum* (36.8% rate in callus and 24.1% rate in somatic embryogenesis) had the highest variation frequency, while *Oryza sativa* (18.4% rate in callus and 12.5% rate in somatic embryogenesis) and *Zea mays* (22.6% rate in callus and 15.3% rate in somatic embryogenesis) had the lowest. In all species, callus-derived plants had the highest frequencies of somaclonal variation, whereas organogenesis had the lowest frequency.

Table 4. Somaclonal variation frequencies across selected species

Plant Species	Variation Frequency in Callus (%)	Variation Frequency in Organogenesis (%)	Variation Frequency in Somatic Embryogenesis (%)
Rice	18.4	9.7	12.5
Maize	22.6	11.2	15.3
Sugarcane	36.8	19.5	24.1
Banana	41.5	21.3	27.6

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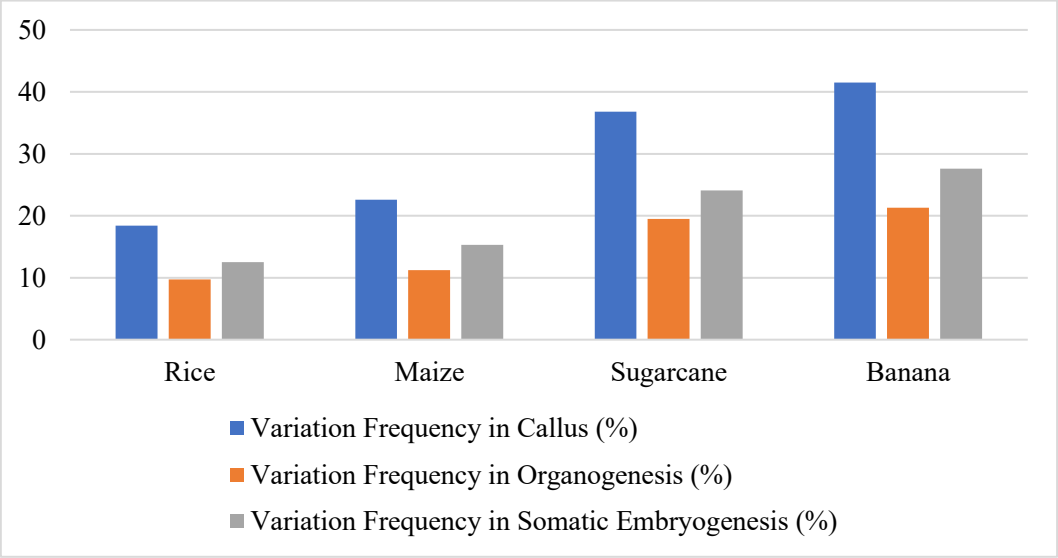

Figure 4: Graphical Representation of Somaclonal variation frequencies across selected species

This trend indicates that polyploid crops like *Saccharum officinarum* and *Musa* spp. have an increased risk of developing variability in tissue culture, likely because of their complex genome structure and better tolerance for changes in ploidy. In contrast, diploid cereals such as *Oryza sativa* and *Zea mays* are more stable. This highlights the need to develop tissue culture techniques for specific species with regard to their genomic context, while factoring in clonal stability and the opportunity to use variation in somaclonal fields for crop improvement.

5. DISCUSSION

The findings suggest tissue culture induces considerable genome-level and epigenetic changes affected mainly by the pathway of regeneration and the complexity of the species genome. Highest genomic instability was executed in callus cultures accompanied by significant chromosomal aberrations, while lower rates of change and even lower rates of instability were observed after organogenesis or somatic embryogenesis. Epigenetic changes were shown primarily by hypomethylation in callus cultures, which resulted in activation of transposons. Finally, somaclonal variation varied across species where polyploid variation was more extreme than di-ploids or cereals indicating the importance of the tissue culture protocol in determining types of species and ploidy to optimize potential variability and clonal stability.

- **Importance:** The research provides a complete evaluation of genome-level and epigenetic variation related to different tissue culture pathways, highlighting the interaction among regeneration method, genome complexity, and epigenetic reprogramming. It highlights that callus culture has the potential for high variation in new traits, but the risk in genetic fidelity, while organogenesis is more appropriate for heirloom traits suitable for clonal propagation.
- **Limitations:** The study was limited to four economically important species and the results may not apply across all plant taxa. Complete DNA methylation was assessed, but did not look at locus-level resolution, which could have provided more information about gene-level epigenetic changes. Environmental factors such as light intensity and media content were controlled, but may have still contributed to the variation observed.

- **Implications:** The findings indicate the necessity of using appropriate tissue culture methods which are optimal based on the desired trade-off between stability and variation. The finding will help inform crop improvement program, micropropagation procedure and breeding strategy where germplasm with beneficial somaclonal variation can be utilized while not adding unwanted genetic and epigenetic variation. Future work are desirable to investigate locus specific epigenetic changes and long-term heritance of tissue culture variation.

6. CONCLUSION

This research illustrates that plant tissue culture produces considerable variability in genomic and epigenetic features, with the degree of change influenced by the intensity of the regeneration route and genome complexity of the species. Callus cultures had the greatest level of genomic instability, DNA hypomethylation, and transposon activation, and thus, somaclonal variation, was elevated even more than with polyploid crops on a low DNA methylation origin level via the induction of chromatin burst in both banana and sugarcane. In contrast, organogenesis presented a better option with more genomic and epigenetic stability of cultivar-and-strain properties while somatic embryogenesis offered intermediate efficacy to public breeder varieties and was able to balance enough variability to be efficient in regeneration and breeding. The data shows that tissue culture routing methods affect the genetic and epigenetic interests on the crops as they will vary tremendously based on the crops performed on callus tissue, even if their ploidy levels are similar. While callus culture can be important for developing valuable traits in supported crops, the genomic-epigenetic stability and somaclonal variation factories create impediment to genetic fidelity. The results recognize variability in post-culture strategies via species-specific genome characteristics and the degree of genomic stability derived from the ploidy of new phenotypic traits of interest in the regeneration process. Future research should identify locus specific epigenetic alterations and the inheritability of induces genome modification traits and introduce a pathway to establish how tissue culture protocols can evaluate somaclonal guitar when traits are advanvanlty seen.

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statistically and, thus, guarantee the soundness and transparency of results. Their general understanding and cooperation were critical in growth of our knowledge on use of tissue cultures to create variability, as well as use within biotechnology on crops to fertilize them, clonal multiplication and biotechnology.

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