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Lady rosetta potato farming: *In vitro* exploration of chitosan and derivatives for multiplication, microtuber induction, and somaclonal variation detection

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This study investigates the efficacy of chitosan and its derivatives, in both regular and nano forms, in promoting the growth and yield of Solanum tuberosum L. 'Lady Rosetta.' The results demonstrate significant improvements compared to the control group. For regular chitosan treatments, shoot length increased markedly. Chitosan lactate at 0.03% produced a maximum shoot length of 16 cm, whereas the control reached only 12.3 cm. Similarly, the highest shoot number (8.3) was observed with N, O-carboxymethyl chitosan at 0.01%, compared to 4.7 shoots in control. Branch number also exhibited enhancement, with chitosan acetate at 0.03% yielding 10 branches relative to 1.7 in the control. Additionally, leaf count per shoot was highest with chitosan lactate (0.03%) at 14.7, in contrast to 6.7 in control. Chlorophyll content significantly increased in treated plants. Chitosan lactate (0.03%) achieved 28.2 mg/g chlorophyll a, while chitosan acetate (0.01%) reached 23.7 mg/g chlorophyll b, compared to 23.5 mg/g and 11.4 mg/g in control, respectively. Moreover, carotenoid content was elevated, with N,O-carboxymethyl chitosan (0.03%) and chitosan lactate (0.03%) recording 7.3 mg/g and 7.14 mg/g, respectively, versus 6.6 mg/g in control. Microtuberization performance was notably enhanced. Chitosan lactate (0.01%) produced up to 25.3 microtubers, significantly exceeding the control (7.7 microtubers). Regarding microtuber size, chitosan lactate (0.03%) yielded microtubers measuring 0.9 cm, compared to 0.4 cm in the control. In nano-treated groups, nano chitosan lactate (0.01% and 0.03%) generated 12.7 microtubers with an average size of 0.4 cm, whereas the control produced 8.3 microtubers at 0.3 cm. Genetic stability was confirmed through RAPD and ISSR analyses, ensuring the absence of somaclonal variations.

Keywords: Chitosan; Chitosan derivatives; Nanobiotechnology; Somaclonal variation detection; RAPD; ISSR

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INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are propagated vegetatively, enabling regeneration from tubers or tuber segments, commonly termed "seed potatoes" or "minitubers." In Egypt, potato cultivation occupied approximately 436,727 feddans during the 2022/2023 season, producing an estimated 5,629,838 tons (Economic Affairs Sector, 2023). To maintain disease-free production systems, Egypt imports 135,000 tons of certified minitubers annually for $261,736,033—a recurring expenditure representing 83% of the sector's seed-related income. Meristem culture offers a sustainable alternative for producing virus-free planting material. This technique involves isolating meristematic tissue from infected plants and culturing it on optimized media, yielding pathogen-free plantlets suitable for large-scale micropropagation (Azad et al., 2020). Such approaches could reduce dependency on imported seed stocks while improving yield stability.

Chitosan, a deacetylated chitin derivative, exhibits versatile applications across agriculture, medicine, and industry. It is a biostimulant in crop production, enhancing nutrient uptake, stress tolerance, and pathogen resistance (Kanawi et al., 2021; Zhang et al., 2022). Its efficacy stems from its unique physicochemical properties, including biodegradability, biocompatibility, and antimicrobial activity against chitin-containing pathogens (Das et al., 2024). Structural modifications further improve its solubility and bioactivity, expanding its utility. Nanoengineered chitosan derivatives demonstrate enhanced functionality in agricultural systems. Applications include abiotic stress mitigation, precision agrochemical delivery, and postharvest pathogen control (Ingle et al., 2022). Notably, chitosan nanoparticles optimize photosynthetic efficiency, seed germination, and stress resilience while minimizing environmental impact (Zhao et al., 2020). These findings align with research demonstrating that chitosan-based encapsulation improves agrochemical efficacy and reduces ecological toxicity (Maluin & Hussein, 2020).

This study evaluates the impact of chitosan and its derivatives, in both conventional and nanoformulations, on *in vitro* potato multiplication and microtube induction, while assessing genetic stability using molecular markers.

MATERIALS AND METHODS

Minitubers sterilization

The production of disease-free potato minitubers (cv. Lady Rosetta) for this study was conducted under the New Valley Project, supported by the Academy of

Scientific Research and Technology (ASRT). To maintain viability, healthy minitubers were stored at 2–4°C with 80% relative humidity. For tissue culture initiation, minitubers were aseptically processed in a laminar airflow cabinet. They were sectioned into cubic explants, and buds were surface sanitized through sequential sterilization treatments. First, explants were washed with sterile distilled water, followed by immersion in 70% ethanol for 5 minutes and 0.2% mercuric chloride ($HgCl_2$) for 5 minutes. $HgCl_2$ was selected as a broad-spectrum sterilant due to its proven efficacy against bacterial, fungal, and viral contaminants. A critical consideration was achieving surface sterilization without compromising explant viability, as excessive penetration of sterilants can damage meristematic tissues. After sterilization, the buds were rinsed three times with autoclaved distilled water (3–5 minutes per wash) to remove residual sterilizing agents.

Chitosan source

Chitosan Egypt graciously provided Chitosan, which was prepared using their protocol, including demineralization, deproteination, and deacetylation.

Chitosan derivatives, culture medium preparation
Chitosan derivatives preparation

Chitosan derivatives were prepared from chitosan according to the methods described (Li *et al.*, 2007; Miao *et al.*, 2006; Xueqing & Huiping, 2016).

Culture Medium Preparation

The culture medium was prepared using distilled water, sucrose (30 g/L), ¾ MS (Murasnige & Skoog, 1962) (3.3 g/L), kinetin (Kin) (0.5 mg/L), gibberellic acid (GA) (0.5 mg/L), and agar (9 g/L). The pH of the medium was adjusted to 5.8 with 1N HCl or 1N NaOH before autoclave. Chitosan treatments were filtered, sterilized, and incorporated into the autoclaved medium. Different concentrations of chitosan and chitosan derivatives (in both regular and nano forms) were used as treatments as follows: T0 = Control, T1 = 0.01% chitosan, T2 = 0.03% chitosan, T3 = 0.05% chitosan, T4 = 0.01% chitosan acetate, T5 = 0.03% chitosan acetate, T6 = 0.05% chitosan acetate, T7 = 0.01% chitosan lactate, T8 = 0.03% chitosan lactate, T9 = 0.05% chitosan lactate, T10 = 0.01% N,O-carboxymethyl chitosan, T11 = 0.03% N,O-carboxymethyl chitosan, T12 = 0.05% N,O-carboxymethyl chitosan

***In vitro* cultures of potatoes with treatments**

In vitro explants were cultured on media supplemented with three concentrations of chitosan and its derivatives. Cultures were maintained in sterile jars and incubated for 28–30 days per subculture cycle, repeated through multiple passages. All tissue culture procedures were conducted under aseptic conditions within a laminar flow cabinet, which was pre-sterilized using 70% ethanol followed by hydrogen peroxide treatment to maintain a contamination-free environment.

Treatments' effect on chlorophyll & carotenoid content

Pigment quantification was performed following a modified protocol adapted from Lichtenthaler and Buschmann (2001). Fresh leaf samples (0.1 g) were homogenized in 1 mL of 80% acetone and transferred to amber vials to prevent photodegradation. The extraction was enhanced by ultrasonic treatment (50°C, 5 min) followed by centrifugation (10,000 × g, 5 min). Absorbance of the supernatant was measured at three wavelengths: 662 nm (chlorophyll a), 645 nm (chlorophyll b), and 470 nm (carotenoids). Pigment concentrations were calculated using the following equations:

Chlorophyll a = 12.7 A662 – 2.59 A645
Chlorophyll b = 22.9 A645 – 4.7 A662
Carotenoids = (1000 A470 – 1.82 chlorophyll a – 85.02 chlorophyll b) / 198

Microtuberization and treatments

Microtuber induction was performed using a modified Donnelly medium (Donnelly et al., 2003) supplemented with chlormequat chloride (CCC) in combination with the experimental treatments. The induction medium was dispensed into culture vessels (three replicates per treatment) and maintained under dark conditions at 25±1°C for 40 days. After incubation, microtubers were carefully harvested and transferred to Petri dishes for quantitative assessment. Each treatment was evaluated for: (1) average total microtuber count (2) diameter/size of microtubers.

***In vitro* cultures of potato with nano treatments**

After evaluating the effects of different concentrations of chitosan and its derivatives on the multiplication and microtuber induction stages, the next step was to assess the impact of their nanoparticle forms on the same parameters.

Nano chitosan and derivatives preparation

The ionic gelation process described by Van Bavel et al. (2023) was used to prepare nanoparticles, as shown in Figure 1. Chitosan powder was completely dissolved in 1% (w/v) acetic acid solution. Separately, sodium tripolyphosphate (TPP) was dissolved in deionized water at 0.05% (w/v). The TPP solution was added dropwise to the chitosan solution under continuous high-speed magnetic stirring. Nanoparticles formed spontaneously through TPP-induced ionic gelation. Chitosan derivatives (chitosan acetate, chitosan lactate, and N,O-carboxymethyl chitosan) were prepared following the same protocol, with modification of the solvent to deionized distilled water instead of acetic acid. The synthesized nano chitosan and chitosan derivatives were characterized using Fourier Transform Infrared Spectroscopy (FTIR), zeta potential analysis, and Transmission Electron Microscopy (TEM) according to Khaled et al. (2023).

Nano treatments and potato multiplication

Murashige and Skoog (MS) medium was prepared according to standard protocols. Nano chitosan and its derivatives were incorporated into the medium at identical concentrations, and the codes used in previous experiments were immediately after preparation. Five biological replicates were established per treatment. Cultures were maintained for 28-30 days under controlled conditions, with all growth parameters recorded and analyzed using SPSS software (version 26; IBM Corp.) with one-way ANOVA and Tukey's post-hoc test ($p<0.05$).

Nano treatments and potato microtuberization

Microtuber induction medium containing chlormequat chloride (CCC) was used, and cultures were incubated in dark conditions for 40 days**.** Microtubers were harvested after incubation, and data were analyzed using SPSS.

Somaclonal variation detection

RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeat) PCR techniques for detecting somaclonal variation are well justified due to their efficiency in rapidly detecting genetic differences in plant populations. These PCR-based methods do not require prior sequence information, making them ideal for large-scale screening in somaclonal variation studies. RAPD broadly employs random primers covering the genome, while ISSR targets highly variable microsatellite regions. Together, they provide a comprehensive assessment of genetic polymorphism and effectively identify somaclonal variation through differences in banding patterns, while also confirming genetic stability in commercial micropropagation. Genomic DNA was extracted from control and treated explants (shoots and leaves) using a plant DNA preparation kit (Jena Bioscience). PCR amplification was conducted using specific primers: A10 (P1) 5'-GTGATCGCAG-3', D5 (P2) 5'-TGAGCGGACA-3' (Rusinowski & Domeradzka, 2012), and Z11 (P3) 5'-CTCAGTCGCA-3' (El_Komy, 2011) for RAPD; and HB10 (P4) 5'-GAGAGAGAGAGACC-3', HB11 (P5) 5'-GTGTGTGTGTGTCC-3', and HB12 (P6) 5'-CACCACCACGC-3' for ISSR amplification (Radwan, 2013). PCR was performed using Taq DNA polymerase. The amplification protocol consisted of initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 92°C for 1 min, annealing at 44°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 10 min and hold at 4°C. Amplified products were separated by electrophoresis on 0.5% agarose gels alongside a DNA ladder.

Statistical analysis

A two-way analysis of variance (ANOVA) with replication was performed to detect significant differences ($P < 0.05$). Tukey's honestly significant difference (HSD) post hoc test was subsequently applied for multiple comparisons between treatment means. All statistical analyses were conducted using SPSS software (version 26; IBM Corp.).

RESULTS

Effect of chitosan and its derivatives on *in vitro* multiplication of potato (*Lady Rosetta*)

Objective: To evaluate the influence of various concentrations of chitosan and its derivatives on potato cv's *in vitro* multiplication rate*.* Lady Rosetta across three subcultures.

Results: From Figures 2 and 3, in the first subculture, excessive root development led to the exclusion of treatments T1, T2, T3, and 0.05% N,O-carboxymethyl chitosan (T12) due to poor growth. Treatments with chitosan acetate (0.01% and 0.03%) showed comparable shoot lengths, while the control (T0) produced the highest number of shoots, a result that was significantly different ($P < 0.05$) from the other treatments. From Figures 2 and 4, in the second subculture, 0.01% chitosan lactate (T7) achieved the longest shoot length, significantly surpassing other treatments ($P<0.05$), and 0.03% chitosan acetate (T5) resulted in the highest number of shoots, which

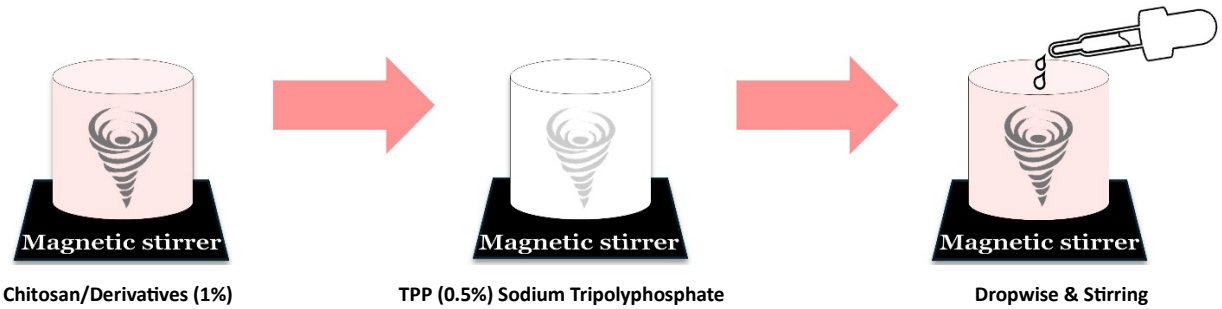

Figure 1. Method for Preparing Nanoparticles from Chitosan and Its Derivatives.

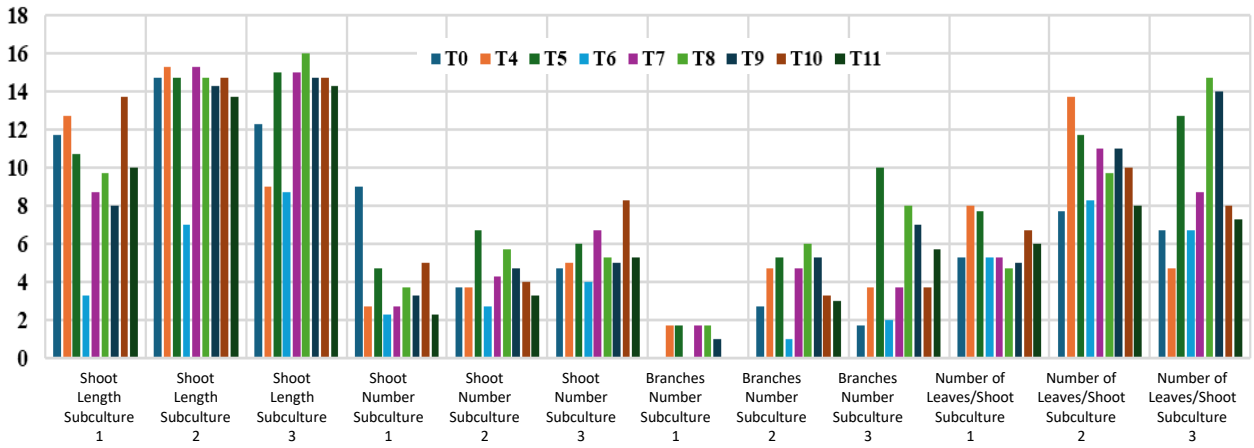

Figure 2. Effect of chitosan and derivatives on *in vitro* multiplication of potato over three subcultures.

was also substantially different from the rest ($P < 0.05$). The highest number of leaves per shoot was observed in chitosan acetate treatments (0.01% and 0.03%), showing significant differences ($P<0.05$) compared to other treatments. From Figures 2 and 5, in the third subculture, 0.03% chitosan lactate (T8) exhibited the maximum shoot length, with significant differences ($P<0.05$) from other treatments. 0.01% N,O-carboxymethyl chitosan (T10) and 0.01% chitosan lactate (T7) produced the highest number of shoots. In comparison, 0.03% chitosan acetate (T5) yielded the most branches and a significantly higher number of leaves per shoot, both demonstrating significant differences ($P < 0.05$) from other treatments.

Conclusion: Chitosan derivatives significantly influenced plant growth parameters, including branching, shoot elongation, and shoot proliferation. Notably, 0.03% of chitosan lactate markedly enhanced development in the third subculture. These findings offer valuable insights into future tissue culture studies and underscore the critical role of selecting optimal chitosan types and concentrations to enhance plant growth efficiency.

Pigment content in response to chitosan and its derivatives treatments

Objective: To determine the impact of chitosan and its derivatives on chlorophyll a, chlorophyll b, and carotenoid content in potato leaves.

Results: As shown in Figure 6, chitosan lactate (0.03%, T8) and N,O-carboxymethyl chitosan (0.03%, T11) exhibited the highest chlorophyll concentration, followed by chitosan acetate (0.01%, T4), chitosan lactate (0.01%, T7), and N,O-carboxymethyl chitosan (0.01%, T10). For chlorophyll b, chitosan acetate (0.01%, T4) contained significantly higher levels, with chitosan lactate (0.03%, T8), N,O-carboxymethyl chitosan (0.03%, T11), and chitosan lactate (0.01%, T7) following in descending order. Finally, the highest carotenoid content was observed in N,O-carboxymethyl chitosan (0.03%, T11) and chitosan lactate (0.01%, T7).

Conclusion: Chitosan enhances photosynthetic pigment accumulation in plants. Our results demonstrate that chitosan lactate (0.03%) and N,O-carboxymethyl chitosan (0.03%) were the most effective treatments for increasing chlorophyll a content. In the case of chlorophyll b, chitosan acetate

Figure 3. Effect of chitosan and its derivatives on potato multiplication in the first subculture.

(0.01%) showed the highest accumulation, whereas N,O-carboxymethyl chitosan (0.03%) also exhibited superior performance in carotenoid production. These findings indicate that carefully selected chitosan derivatives and concentrations can significantly improve chlorophyll and carotenoid levels in plant systems.

Microtuberization under chitosan and its derivatives treatments

Objective: To assess the role of chitosan and its derivatives in promoting microtuber formation under *in vitro* conditions.

Result: As evidenced by Figures 7-9, chitosan lactate treatments (0.01% T7 and 0.03% T8) produced significantly larger microtubers compared to other treatments ($p < 0.05$). These results indicate that both concentrations effectively promote microtuber development, with T8 yielding microtubers of greater

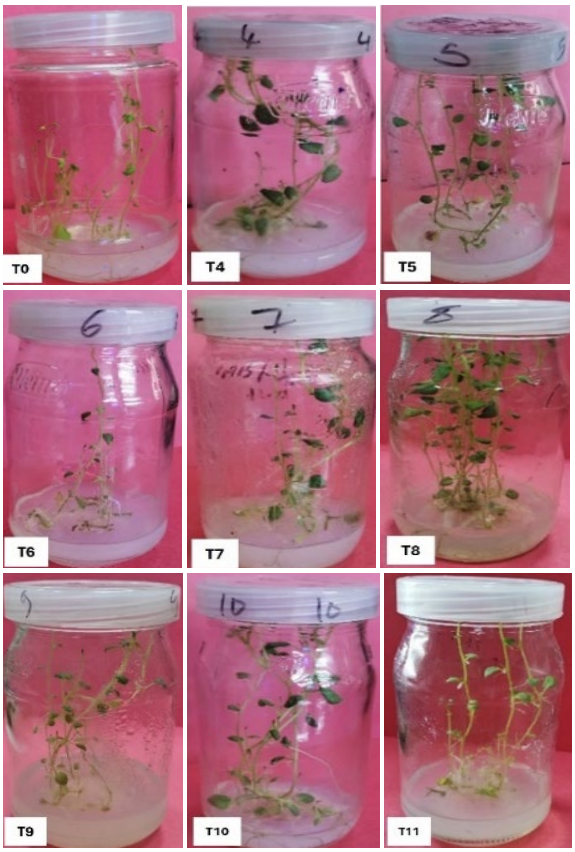

Figure 4. Effect of chitosan and its derivatives on potato multiplication in the second subculture.

average diameter (0.8* ± 0.3 cm) than T7 (0.7 ± 0.4 cm). This size enhancement suggests improved tuber quality and developmental potential under chitosan lactate treatment.

Conclusion: This study demonstrates chitosan lactate's potential as an effective biostimulant for microtuber cultivation, significantly enhancing tuber size and yield potential. The observed diameter increases (50% greater than controls) suggest substantial crop quality and productivity improvements. While these results highlight chitosan lactate's agricultural value, further research should be investigated:

- Molecular mechanisms underlying tuber enlargement
- Field-scale validation of growth enhancements
- Economic feasibility for commercial production

Characterization of nano chitosan and its derivatives

Objective: The synthesized nanochitosan and its derivatives were characterized using Fourier-transform infrared spectroscopy (FTIR) to analyze functional groups, transmission electron microscopy

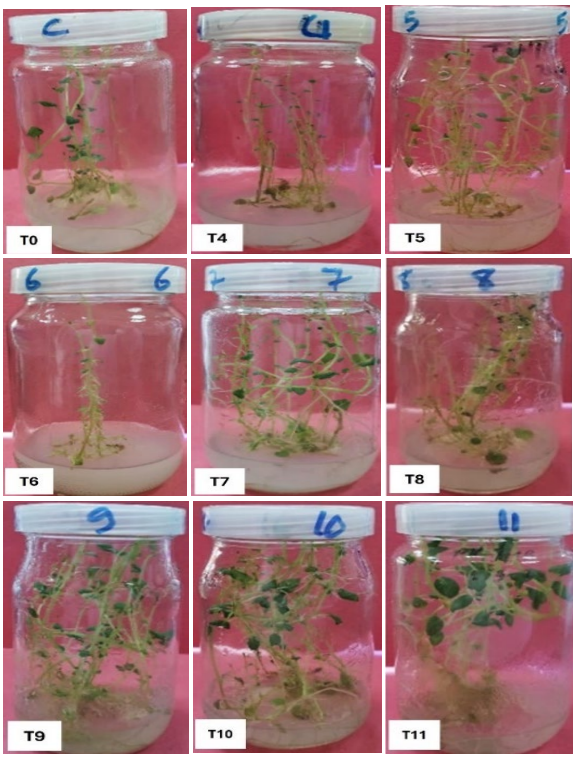

Figure 5. Effect of chitosan and its derivatives on potato multiplication in the third subculture.

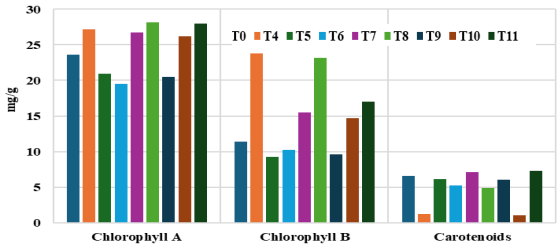

Figure 6. Effect of treatments on chlorophyll a, chlorophyll b, and carotenoid content.

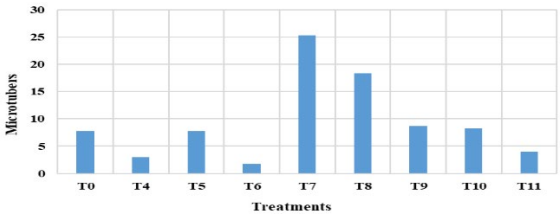

Figure 7. Number of microtubers under different treatments.

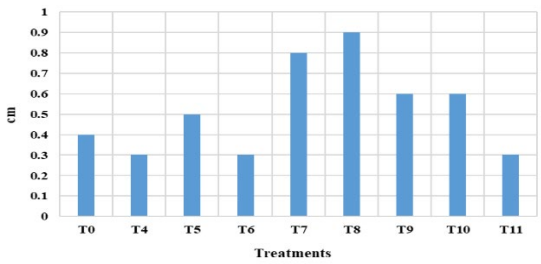

Figure 8. Sizes of microtubers under various treatments.

(TEM) for morphological evaluation, and zeta potential measurements to assess colloidal stability.

Results: Fourier Transform Infrared (FTIR) Spectroscopy: FTIR analysis confirmed characteristic functional groups in nanochitosan and its derivatives (Figure 10). The broad absorption bands between 3363.25 cm^{-1} and 3735.44 cm^{-1} correspond to O-H stretching vibrations of hydroxyl groups. Distinct peaks at 3351.68–3363.25 cm^{-1} in nanochitosan acetate, lactate, and carboxymethyl derivatives represent N-H stretching of primary amines. Prominent absorption bands in the 1540.85–1644.98 cm^{-1} range indicate amide I (C=O stretch) and amide II (N-H bend) vibrations, confirming ionic interactions between chitosan protonated amino groups (NH_3^+) and tripolyphosphate anions. Characteristic C-O-C stretching vibrations appeared between 1243 cm^{-1} and 1189 cm^{-1}.

Transmission Electron Microscopy (TEM): TEM imaging revealed that all chitosan-based nanoparticles were generally spherical with a narrow size distribution (Figure 11). **Nano chitosan** (a) showed the smallest average size at (23 nm), followed by **nano chitosan acetate** (b) (40 nm), **nano carboxymethyl chitosan** (d) (35 nm), and the largest particles were observed in **nano chitosan lactate** (c) at (130 nm). These differences in particle size may result from the varying chemical modifications, which influence molecular packing and aggregation behavior during nanoparticle formation.

Zeta Potential Analysis: Zeta potential measurements demonstrated that most nano chitosan formulations were positively charged, indicating good colloidal stability (Figure 12). **Nano chitosan** (a), **chitosan acetate** (b), and **carboxymethyl chitosan** (d) exhibited zeta potentials of +37.7 mV, +26.1 mV, and +12.5 mV, respectively, suggesting strong electrostatic repulsion and reduced risk of nanoparticle aggregation. In contrast, **nano chitosan lactate** (c) displayed a negative zeta potential of –5.71 mV, also indicating stability through repulsion, likely due to the presence of ionized carboxylate groups. The surface charge variation reflects the chemical nature of each derivative and confirms the successful synthesis of stable nano-formulations.

Conclusion: FTIR analysis confirmed the successful incorporation of functional groups in nano chitosan derivatives, suggesting diverse applications. TEM results show predominantly spherical nanoparticles with size variations due to specific chemical modifications, potentially affecting performance. Zeta potential measurements indicated good colloidal stability, essential for biomedical and pharmaceutical

uses. Overall, the findings highlight the favorable characteristics of synthesized nano chitosan and its derivatives for targeted applications, particularly as effective carriers in drug delivery systems.

Nano Chitosan and Its Derivatives' Treatments on Potato Multiplication

Objective: To investigate the effects of nano chitosan and its derivatives on *in vitro* multiplication.

Results: From figures 13 and 14, Nano chitosan lactate 0.01% (T7) had the highest shoot length among other treatments ($P < 0.05$). Regarding the number of shoots, nano chitosan 0.01% (T1) had the greatest number of shoots, which was significantly different from the rest of the treatments, whereas nano chitosan lactate 0.05% (T9) had the least. The number of branches was at its maximum using nano chitosan acetate 0.01% (T4), which had significantly more branches, followed by nano chitosan lactate 0.03% (T8). Nano N,O-carboxymethyl chitosan 0.03% (T11) had the most leaves per shoot (16 leaves/shoot). Statistical analysis shows significant differences between the treatments, which are worth noting for future research.

Conclusion: The positive effects observed suggest that nano chitosan and derivatives can improve growth rates, propagation efficiency, and overall viability of plant tissues in a controlled environment. This study highlights the importance of exploring innovative biopolymers like nano chitosan in tissue culture applications, paving the way for advancements in plant propagation techniques and agricultural practices.

Nano Treatments on Microtuber Formation

Objective: Evaluate microtuberization efficiency under nano chitosan and derivative treatments.

Results: From Figures 15, 16, and 17, the treatments with nano chitosan lactate 0.01% (T7) and nano N,O-carboxymethyl chitosan 0.01% (T10) had the highest number of microtubers, which was significantly different from the other treatments. Additionally, among all the treatments, nano chitosan lactate with 0.03% (T8) had the significantly largest microtubers, while nano chitosan 0.01% (T1) was the least.

Conclusion: The results demonstrate that nano chitosan lactate at 0.01% (T7) and nano N,O-carboxymethyl chitosan at 0.01% (T10) significantly increased the number of microtubers. Additionally, nano chitosan lactate at 0.03% (T8) produced the largest microtubers, while nano chitosan at 0.01% (T1) yielded the least. These findings suggest that optimizing chitosan formulations can effectively enhance microtuber production in agriculture.

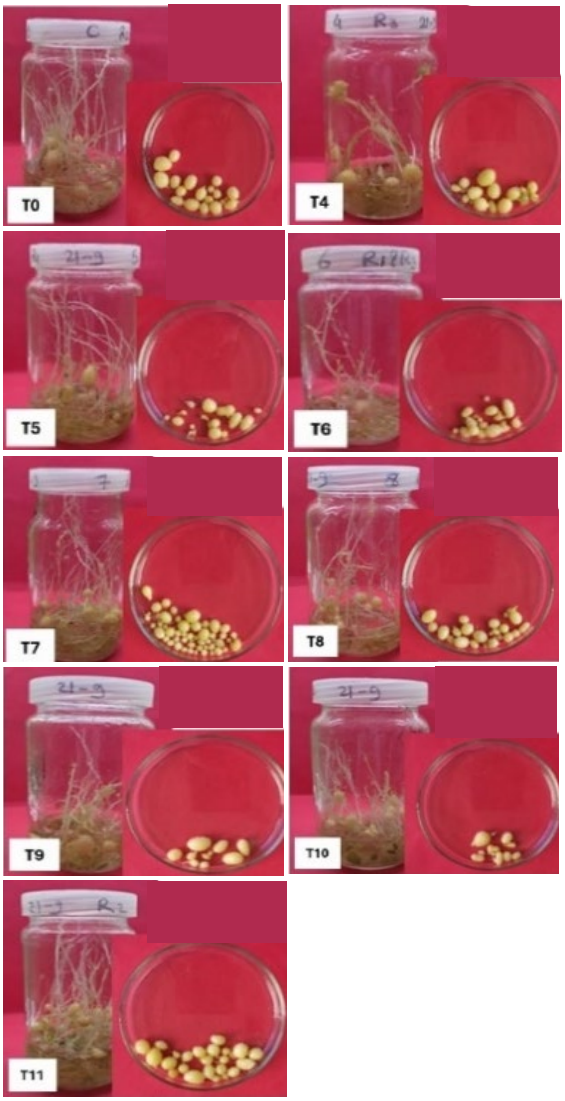

Figure 9. Microtuberization with treatments.

Somaclonal variation assessment

Objective: To evaluate the genetic stability of chitosan-treated plants using molecular marker analysis.

Results: From Figure 18, both analyses revealed consistent monomorphic banding patterns across all treated plants, demonstrating absence of detectable somaclonal variation induced by chitosan or its derivative treatments.

Conclusion: Genetic stability assessment using molecular markers confirmed that chitosan and its derivatives induced no detectable somaclonal variation in treated plants. The monomorphic banding patterns across all samples demonstrate preserved genetic integrity, indicating that these compounds are safe for plant development applications without risking genetic alterations.

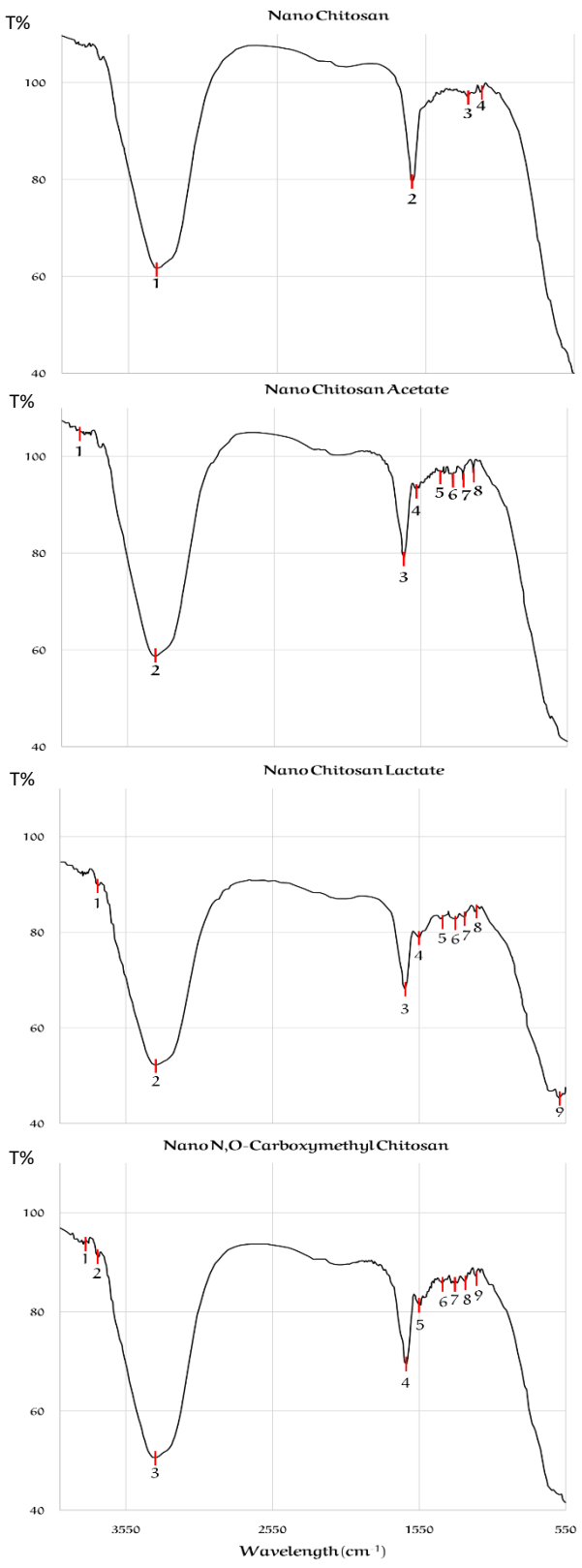

Figure 10. FTIR analysis of nano chitosan and derivatives.

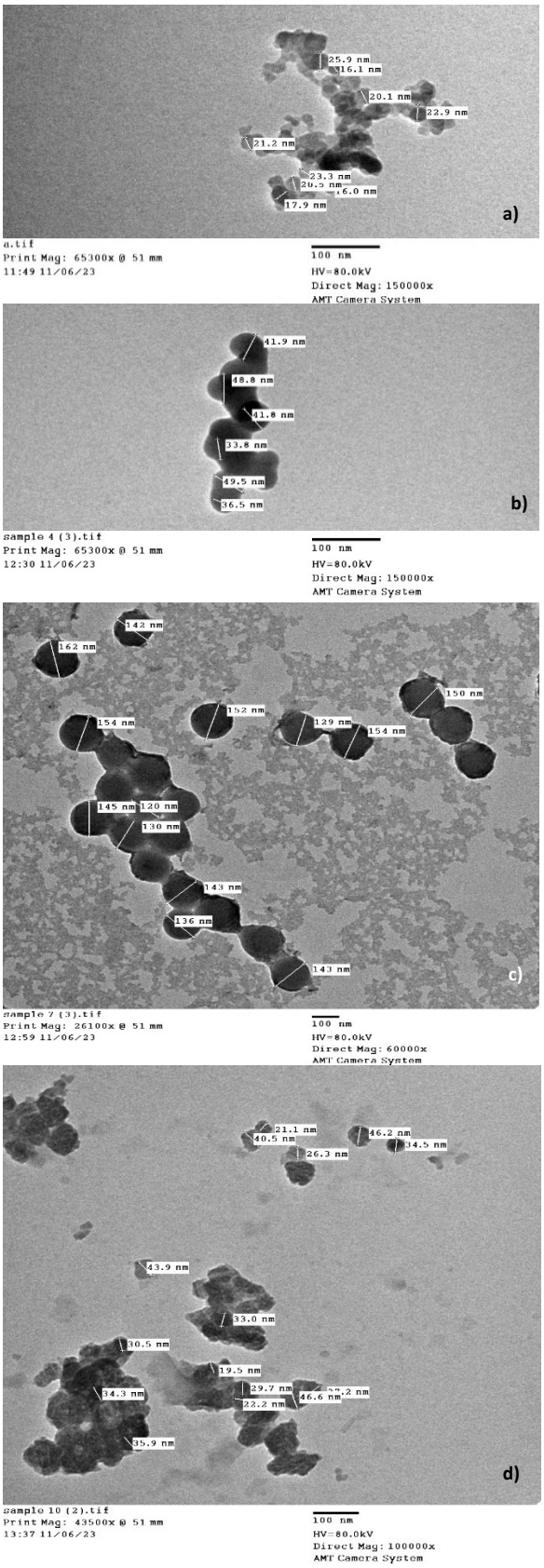

Figure 11. TEM images of synthesized nanoparticles.

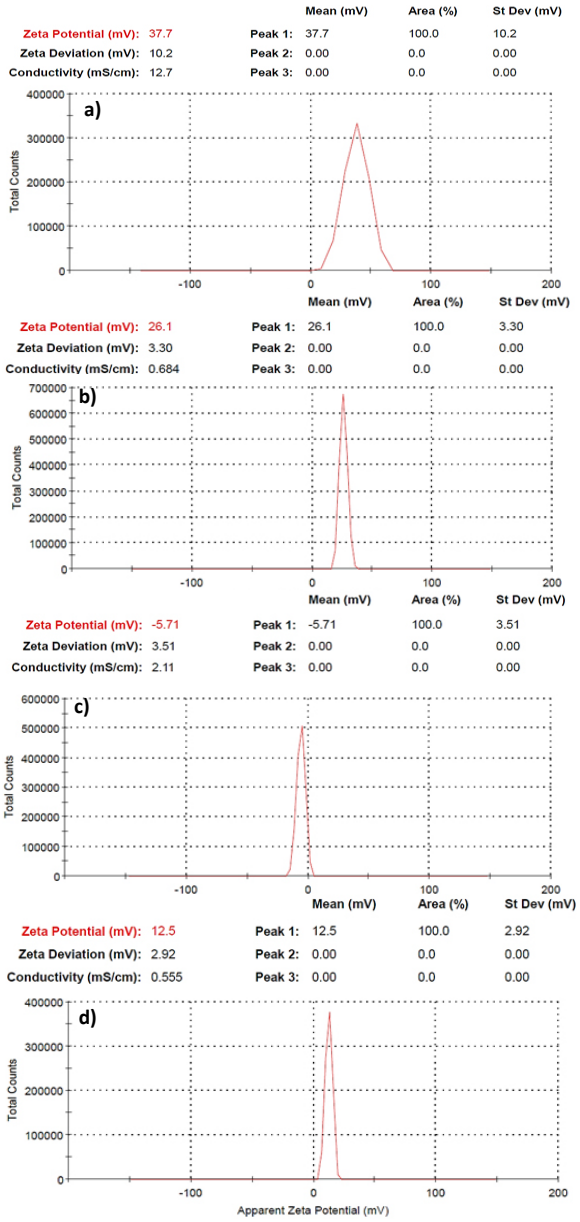

Figure 12. Zeta potential of nanoparticle formulations.

Results: From Figure 18, both analyses revealed consistent monomorphic banding patterns across all treated plants, demonstrating absence of detectable somaclonal variation induced by chitosan or its derivative treatments.

Conclusion: Genetic stability assessment using molecular markers confirmed that chitosan and its derivatives induced no detectable somaclonal variation in treated plants. The monomorphic banding patterns across all samples demonstrate preserved genetic integrity, indicating that these compounds are safe for plant development applications without risking genetic alterations.

DISCUSSION

Chitosan and its derivatives have emerged as promising biostimulants in plant tissue culture owing to their biocompatibility, organic origin, and growth-promoting properties. This study systematically evaluated various concentrations of chitosan derivatives (standard and nanoformulations) during micropropagation and microtuber induction in Solanum tuberosum L. cv. Lady Rosetta. Molecular analyses confirmed the absence of treatment-induced somaclonal variations across all experimental groups.

Effect on vegetative growth

Regular chitosan treatments (0.01% T1, 0.03% T2, 0.05% T3) induced exclusive white root formation, attributable to acetic acid solvent effects. Consequently, these groups were omitted from microtuberization studies. This contrasts with previous studies using water-soluble chitosan formulations (ChitoPlant®) (Kowalski et al., 2006; Asghari-Zakaria et al., 2009), underscoring the critical role of chitosan solubility and solvent selection *in vitro* performance. Among regular derivatives, 0.03% chitosan acetate (T5) significantly increased branch formation ($P < 0.05$ versus control).

While chitosan acetate applications in potato micropropagation remain scarcely documented, parallel dose-dependent responses have been reported in Lavandula officinalis (Atteya et al., 2023), where optimal growth enhancement occurred at 2–3 g/L foliar applications, with diminished effects at higher concentrations. Chitosan lactate treatments (T7-T9) demonstrated significant growth-promoting effects, with T7 increasing microtuber number ($P < 0.05$) and T8-T9 enhancing leaf development These results align with Steglińska et al. (2024), who reported improved chlorophyll content, gas exchange parameters, photosynthetic activity, and stress tolerance in chitosan-treated potato cultures, indicating enhanced physiological performance. N,O-carboxymethyl chitosan (NOCC) at 0.01% (T10) significantly increased shoot numbers ($P<0.05$), demonstrating potential for shoot proliferation. Consistent with these findings, Wu et al. (2024) showed that O-CMC-NPs enhanced drought tolerance in maize through increased chlorophyll content and antioxidant enzyme activity ($P < 0.01$), further supporting the efficacy of NOCC formulations.

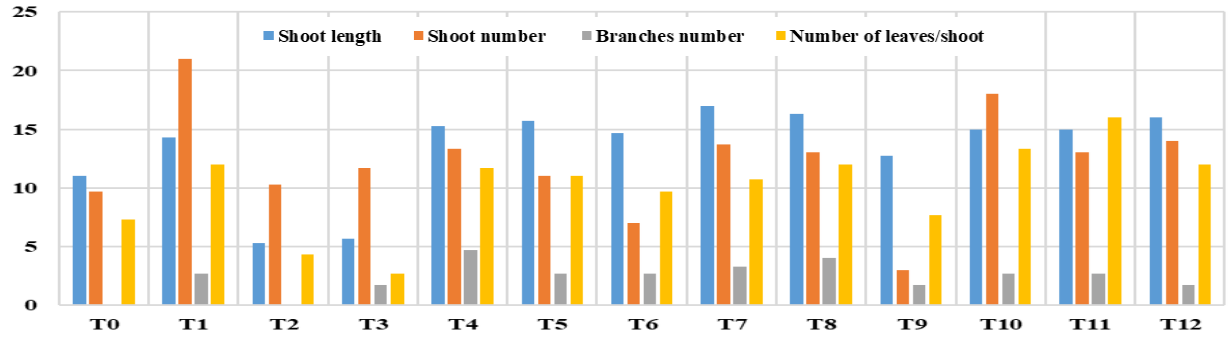

Figure 13. Multiplication under nano chitosan and derivative treatments.

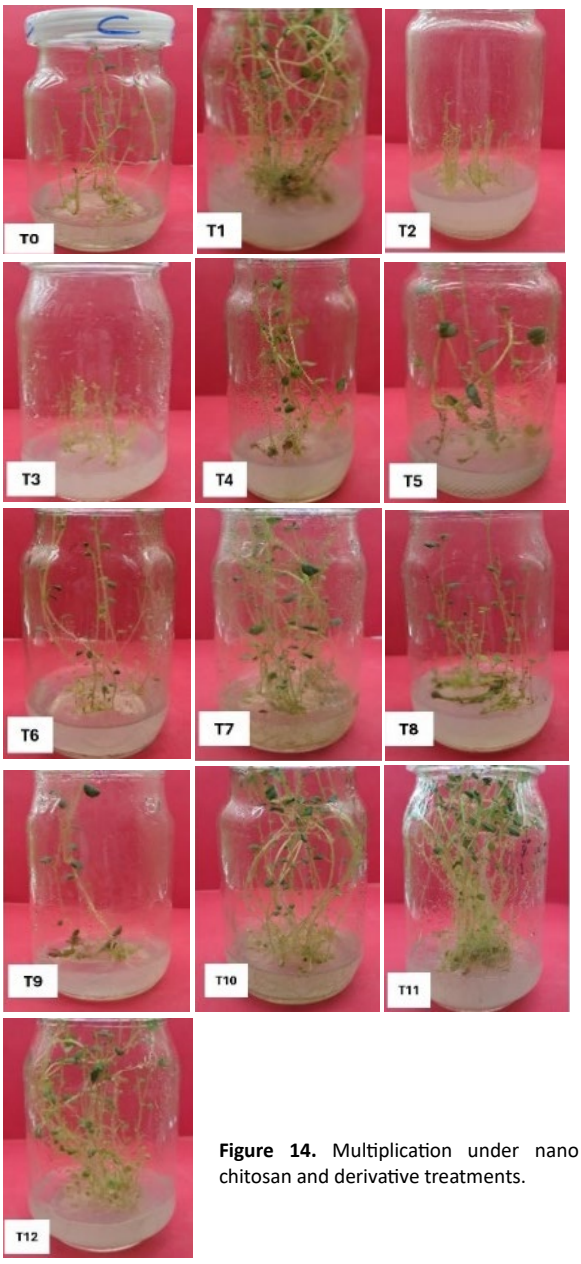

Figure 14. Multiplication under nano chitosan and derivative treatments.

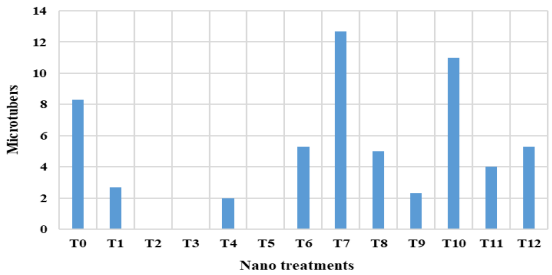

Figure 15. Number of microtubers formed under nano treatments.

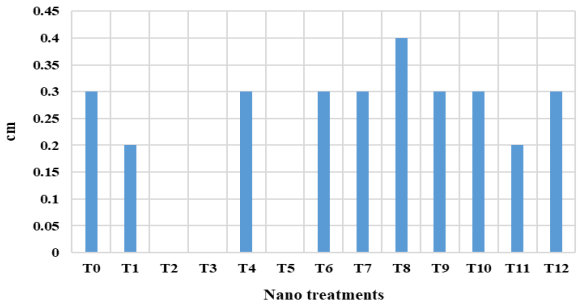

Figure 16. Sizes of microtubers formed under nano treatments.

Effect of nanoparticles

Applying chitosan nanoparticles generally produced more variable, yet often improved, results than their regular-sized counterparts. This may be attributed to the dilution effect of tripolyphosphate (TPP), which was used as a crosslinking agent and may reduce nanoparticle bioactivity. Nano-chitosan (T1) at 0.01% significantly improved shoot number and vigor ($P < 0.05$), demonstrating its effectiveness for microtuberization. These findings partially align with Shebl et al. (2022), who reported that 0.4 g/L nano-chitosan produced suboptimal morphology with short, poorly branched plantlets. In our study, the lower concentration (T1) yielded better morphological outcomes, potentially due to cultivar differences or reduced toxicity at this concentration.

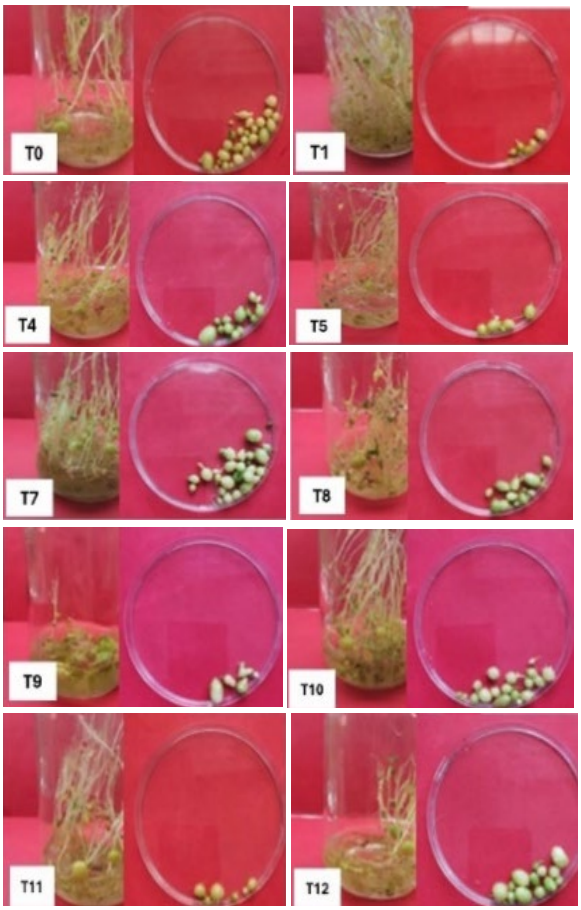

Figure 17. Microtubers formed under nano treatments

Similarly, nano-chitosan lactate (T7, T8), particularly T8 (0.03%), showed statistically significant increases in both shoot length and microtuber size ($P < 0.05$). Nano-NOCC (T10, T11) enhanced shoot regeneration and microtuber formation *in vitro*, producing the highest shoot numbers, though these differences were not statistically significant. These treatments also improved leaf numbers and microtuber counts.

Effects on pigment content

This study demonstrated that chitosan and its derivatives significantly enhanced plant growth and photosynthetic pigment accumulation. Treatment T8 (0.03% chitosan lactate) and T11 (0.03% nano-NOCC) yielded the highest chlorophyll a content, while T4 (0.05% chitosan acetate) and T8 produced significantly elevated chlorophyll b levels ($P<0.05$). T11 and T7 (0.01% nano-chitosan lactate) showed the highest content of carotenoids, though without statistical significance. The biostimulant effects were further evidenced by significant improvements in plant height, leaf area, chlorophyll content ($P<0.05$) across multiple treatments.

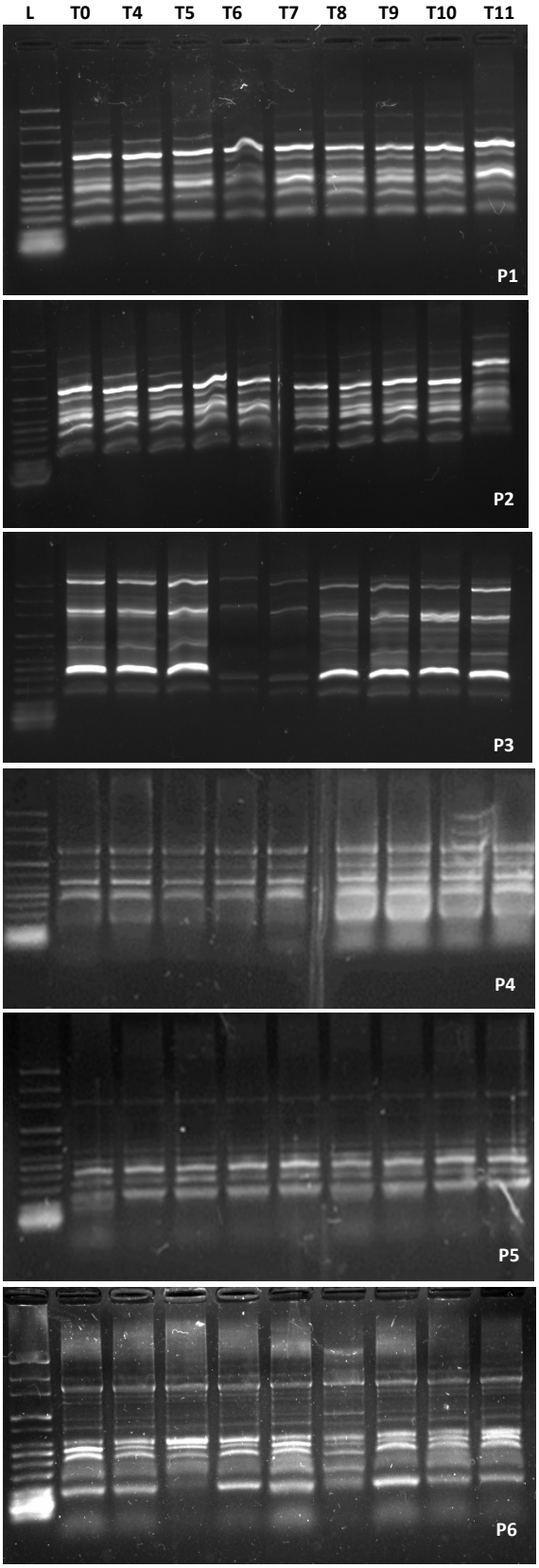

Figure 18. RAPD and ISSR profiles of treated and control plantlets.

These results align with Khairy et al. (2022), who reported that nano-chitosan (100 ppm) effectively suppressed bacterial wilt in solanaceous crops ($P < 0.05$), and Zheng et al. (2021), where chitosan applications (0.25–0.5 g/L) significantly mitigated late blight severity in potatoes, reinforcing the dual role of chitosan derivatives as both growth enhancers and protective agents.

Genetic stability

Somaclonal variation was not observed in any treatments, and no morphological abnormalities were detected in regenerated plantlets, confirming the genetic stability of chitosan and its derivatives. These results are consistent with Al-Mokadem et al. (2022), who demonstrated that chitosan combined with phosphorus alleviated potato virus Y stress without causing morphological deviations, further supporting chitosan's safety in tissue culture applications. In conclusion, our study represents a pioneering investigation of chitosan acetate, chitosan lactate, and NOCC in potato tissue culture. Given the limited research on these specific compounds, further studies are needed to fully explore their potential benefits and applications. Continued research and application of these biopolymers will advance potato tissue culture techniques and promote sustainable agricultural practices.

SUMMARY

This study investigated the effects of chitosan and its derivatives, including both conventional and nanoparticle formulations, on the *in vitro* culture of *Solanum tuberosum* L. cv. Lady Rosetta, with a focus on plant growth, microtube induction, and genetic stability. The results revealed that low concentrations of chitosan derivatives, particularly chitosan lactate and nano-N,O-carboxymethyl chitosan (nano-NOCC), significantly enhanced shoot regeneration, microtuber formation, and stress tolerance. Among the treatments, 0.03% chitosan lactate (T8) and 0.03% nano-NOCC (T11) yielded the highest chlorophyll *a* content, whereas 0.05% chitosan acetate (T4) and T8 significantly increased chlorophyll *b* levels. Although T11 and T7 (0.01% nano-chitosan lactate) exhibited the highest carotenoid content, the differences were not statistically significant. These findings support the hypothesis that chitosan derivatives positively influence photosynthetic activity, consistent with existing literature on chitosan's role in enhancing chlorophyll and carotenoid accumulation. Nanoparticle formulations generally induced more variable but often superior responses than conventional chitosan. Notably, 0.01% nano-chitosan (T1) significantly increased shoot number and vigor, while nano-chitosan lactate (T7, T8) improved shoot elongation and microtube size. These results align with prior studies demonstrating that chitosan nanoparticles enhance plant growth and stress resilience. No somaclonal variation was detected in any treatment, confirming the genetic stability of regenerated plants. This indicates that chitosan and its derivatives do not compromise genetic integrity in Solanum tuberosum cv at tested concentrations. Lady Rosetta.

In conclusion, this study demonstrates the potential of chitosan derivatives, particularly chitosan lactate and nano-NOCC, as biostimulants in potato tissue culture, whether in conventional or nanoformulations. These findings represent a novel contribution to the field, providing a foundation for further research to optimize formulations, assess efficacy across cultivars, and explore applications under diverse environmental conditions.

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