

## ***In vitro* Propagation of Potato (*Solanum tuberosum* L.) Using Five Different Genotypes Including the Production of Minitubers and Super Elite**

Abdelrahman A. Abdeen<sup>1\*</sup>, H. A. Emar<sup>2</sup>, A. A. Nower<sup>2</sup>, Nermin Gamal<sup>1</sup>, I. A. Ibrahim<sup>2</sup>

<sup>1</sup> Department of Agricultural Biotechnology, Biotechnology College, Misr University for Science and Technology (MUST), Giza, Egypt.

<sup>2</sup> Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt.

DOI: 10.21608/rjab.2025.345956.1059

Received: 23 December 2024

Accepted: 9 January 2025

\*Corresponding author: Abdelrahman Ahmed Abdeen

E-mail: [abdelrahman.mohamed@must.edu.eg](mailto:abdelrahman.mohamed@must.edu.eg)

### **ABSTRACT**

Potato (*Solanum tuberosum* L.) is a vital crop in Egypt, with the country importing approximately 120,000 MT of seed potatoes annually and exporting over 759,200 MT of ware potatoes. This positions Egypt as Africa's leading potato producer. This study investigated the micropropagation of five potato cultivars (Hermes, Spunta, Lady Rosetta, Cara, and Silana) using *in vitro* techniques. Nodes from plants were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of kinetin (KIN) and Naphthalene Acetic Acid (NAA). Results revealed significant differences among cultivars in survival rates, shoots multiplication, and rooting responses. Hermes explants showed the highest survival rate (100%), with lower but statistically similar survival (96.6%) observed for Silana and Hermes on specific treatments. The highest shoot number (13.6 per jar) was recorded for Hermes cultured on 0.25 mg/L KIN, followed by Hermes and Spunta on 0.5 mg/L NAA. Maximum shoot lengths (7.044 cm and 6.908 cm) were observed with Spunta and Hermes on combined KIN-NAA treatments. Hermes on 0.25–0.5 mg/L KIN exhibited the highest leaf numbers, while root number peaked for Cara and Hermes on 0.5 mg/L NAA + 0.1 mg/L KIN. Spunta exhibited the longest roots (9.667 cm) on 1.0 mg/L NAA + 0.5 mg/L KIN. Acclimatization showed high survival rates, with Lady Rosetta and Spunta achieving 96%. Lady Rosetta also produced the highest minituber weight (40.2 g), while Hermes achieved the highest tuber count (10.7 per plant). These findings emphasize cultivar-specific optimization of growth regulators for large-scale, disease-free potato production in Egypt.

**Keywords:** Potato Micropropagation, *In vitro* Culture, Minituber Production, Seed Potatoes, Cultivars Comparison.

### **1. INTRODUCTION**

The potato (*Solanum tuberosum* L.) constitutes a paramount root and tuber crop that serves as a vital source of sustenance for numerous individuals within the nation. It represents an effective strategy for mitigating food insecurity, particularly in scenarios of disaster (Tolessa *et al.*, 2017). Egypt, the largest potato producer in Africa, relies heavily

on the importation of seed potatoes to sustain its domestic production, with imports in 2018 (January–October) reaching approximately 120,000 metric tons (MT). The country is capable of producing around 5 million MT of ware potatoes annually. In 2022, Egypt ranked as the fifth largest global potato exporter, with exports valued at \$357 million (Observatory of Economic Complexity, 2022). Egyptian

potatoes, renowned for their exceptional quality, captured 5% of the global potato export market in 2018, with exports valued at \$259.6 million (Sakara and Badour, 2020; Observatory of Economic Complexity, 2022).

Conventional potato seed production methodologies encompass a series of successive multiplications of the nucleus seed tubers through super elite, elite, and certified seed stages (Ahloowalia, 1999). The quality of seed tubers constitutes a critically essential determinant for the yield of potatoes. Given that it is a vegetatively propagated species, agents of fungal, bacterial, and notably viral diseases are readily transmissible through the tubers. Viral pathogens are predominantly accountable for the degeneration observed, which is typified by a decline in vigor, productivity, and disease resistance of potato cultivars following repeated cultivation from the same tuber lot (Sangar *et al.*, 1993).

Micropropagation is considered a superior alternative to traditional potato propagation methods (Wang and Hu, 1982). This technique can yield thousands of plants within a short temporal span, utilizing specific growth regulators to optimize both shoot and root development (Misal and Chavan, 2024). The *in vitro* environment guarantees that the resultant plants are devoid of pathogens, an aspect that is pivotal for sustaining crop health and yield (Singh *et al.*, 2023). Meristem culture represents a significant application of plant tissue culture aimed at the eradication of viruses from planting materials (Badoni and Chauhan, 2010). Consequently, an appropriate size of shoot tips specific to particular plant species or genotypes must be excised. Shoot tips measuring between 0.5 to 2.0 mm in length may be utilized, contingent upon the genotypes (Wang and Valkonen, 2008). *In vitro* propagation utilizing nodal cuttings is indeed a well-established methodology for the expedited multiplication of potatoes. This approach facilitates the efficient generation of high-quality seed material, markedly

enhancing yields and ensuring the provision of healthy potato plants (Smirnova and Podolian, 2024). Minitubers, generated from *in vitro* plantlets grown in controlled greenhouse environments, have been shown to enhance growth and yield (Gisyuk *et al.*, 2023), with studies highlighting that using micro-tubers and micro-plants in greenhouse settings (Etdzaeva and Oves, 2022), soil, soilless systems like hydroponics and aeroponics, or field conditions can achieve higher productivity than conventional methods (Särekanno, 2011).

The aim of this study is to produce *in vitro* virus free potato plants through micropropagation and production of minitubers and super-elite of potato (*Solanum tuberosum* L.).

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

This study was carried out at plant tissue culture laboratory, plant biotechnology department, Genetic engineering and biotechnology research institute, University of Sadat city, Sadat city, Egypt. The potato sprouts (Cara, Hermes and Spunta) and the *in vitro* cuttings (Lady Rosetta (LR) and Silana) were used as explants. All cultivars of this study were free of potato viruses X, Y and Leafroll. The source of tubers and *in vitro* plants were Provided from University of Sadat city, Sadat city, Egypt.

### 2.2. Preparation and sterilization stage

The potato tuber seeds were sprayed with GA<sub>3</sub> at 1 ppm to break dormancy and then they were stored in the dark for 10-15 days until sprouting occurred. The sprouts were sterilized by soaked in sterilizing solution consists of 20% commercial bleach solution (Clorox 1% NaOCl). Therefore, the sprouts were subjected to shaking for 20 minutes using electronic shaker. The explants were thoroughly washed three times with sterilized distilled water to remove the disinfectant and detergent.

### 2.3. Establishment stage

The Potato sprouts and *in vitro* cutting explants were excised and cultured aseptically in 300ml culture jars each containing 30 ml of solid medium based on MS (Murashige and Skoog, 1962). Strength basal medium with 3% sucrose and 0.8% agar. All these procedures were conducted in the laminar air flow hood. The pH of the medium was adjusted to 5.6-5.8 with 1N of either HCl or NaOH, thereafter they were autoclaved at 121°C under a pressure of 1.5 Kg/cm<sup>2</sup> for 20 minutes. The medium was left to cool and solidify in a sterilized environment after autoclaving. The cultured explants were incubated at 24 ± 2°C for 30 days. The contaminated and survival percentage were recorded. A total of 27 explants of Spunta cv., 24 of Kara cv., and 30 of Hermes cv. were excised. The other cultivars were used as *in vitro* plants. After the regeneration of the new shoots, Regenerated shoots from the establishment stage will be carefully examined, and healthy, vigorously growing shoots will be selected.

Axillary shoot tips 1.5-2.0 mm were isolated aseptically using a sterile scalpel from the upper to middle part of the mother plants according to Kim *et al.*, (2006). These excised shoot tips will be inoculated onto fresh MS medium supplemented with appropriate plant growth regulators to induce shoot multiplication. The resulting shoot cultures were regularly subcultured and propagated to establish a stock of virus-free potato shoots.

### 2.4. Multiplication stage

All cultivars were repropagated with single nodes cutting after 3-4 weeks from establishment stage to study the effect of different growth regulators (Kin, NAA) and the interaction between them with different concentrations (0.0, 0.25, 0.5 and 1.0) mg/l. Nodal segments, each with 1-2 leaves, were separated, and three explants were placed in each jar. The nodal segments were cultured on MS medium containing different treatments of growth regulators (KIN, NAA) as shown in

(Table 1). Four weeks after culturing, surviving rates, number of branches, plant length (cm), number of nodes, number of leaflets, number of roots, rooting percentages and callus percentages per replicate were recorded.

### 2.5. Rooting stage

The obtained shoots of all potato (*Solanum tuberosum* L.) cultivars from the multiplication stages were individually separated and excised into nodal segments then cultured on a rooting medium. The medium contained MS salts, sucrose at 30 g/l supplemented with different concentrations of KIN (0.0, 0.1 and 0.5) and NAA (0.0, 0.5, 1.0, and 2.0) mg/l and the interaction between them as shown in Table (2). Four weeks after culturing, the number of branches, root length (cm) and number of roots per jar were recorded.

### 2.6. Acclimatization and minitubers formation

This stage was performed in the greenhouse of the Genetic engineering and biotechnology research institute, University of Sadat city, Sadat city, Egypt. Plantlets, 30 days old with well-developed shoots and roots, they were carefully removed from the culture vessels using fine forceps. The medium attached to the roots was carefully washed with tap water.

The regenerated plantlets were successfully established *ex vitro* on peat and sand mixture (2:1, V:V) covered with clear plastic sheath (Fig.2). To reduce sudden shock, the pots were kept in the controlled environment in the laboratory. When the plantlets appeared to be self-sustainable, they were then transferred to the field. Then, the growth was observed after 4 weeks and data was collected on shoot number and length, leaves number, nodes numbers, number and length of roots. Four weeks old, hardened plantlets were transplanted to normal fertile soil for minitubers production after four weeks of transferring the plants, number of shoots, number of leaves data were collected, after 90-

120 days minitubers numbers, weight and volume data were collected.

### 2.7. Super elite production

To produce super elite, minitubers were recultured in a private farm to perform superelite seed, After 100 days, data on the number, weight, and volume of super elite seeds were collected. Five potato cultivars (Spunta, Cara, Lady Rosetta, Hermes, and Silana) were initially selected for micropropagation. However, due to insufficient plant material obtained during the acclimatization stage, the Silana cultivar was excluded from subsequent phases of minituber and super elite production.

### 2.8. Statistical analysis.

The experimental data were analyzed using MSTAT-C statistical software (Michigan State University, East Lansing, MI, USA). The experiment was set up as a Randomized Complete Block Design (RCBD) with two factors (Freed *et al.*, 1991). Analysis of Variance (ANOVA) was performed to determine the significance of main effects and interactions (Fisher, 1925). Means were separated using Fisher's Least Significant Difference (LSD) test at  $p \leq 0.05$ . All treatments were replicated at least three times (Fisher, 1935).

## 3. RESULTS

### 3.1. Sterilization and establishment

All cultivars' sprouts were sterilized with the same method, shoot tips were excised and established on MS media free of growth regulators, Spunta cv. gave the highest survival percentage of sterilization with (33.3%), about 9 explants survived from 27 explants. The Cara cultivar had a survival rate of 25%, with 6 explants surviving out of 24. The Hermes cultivar recorded the lowest survival percentage (20%), with 6 explants surviving out of 30 explants cultured.

### 3.2. Shoot multiplication.

A comparison between KIN and NAA, and the interactions between them, resulted in different outcomes for the five cultivars. In Table (3), data showed that the highest significant survival rate (99.57%) was significantly observed in the Hermes cultivar, followed by the Silana and Cara cultivars. The lowest survival rate was recorded with the Spunta cultivar (78.77%). Data for the main treatments effect showed that MS medium containing 0.5 mg/L KIN + 0.25 mg/L NAA resulted in the highest survival rate. Other treatments followed, but there were no significant differences between them. Regarding the interaction, a 100% survival rate was observed with some combinations of cultivars and treatments, followed by other combinations that showed no significant difference.

In Table (4), data showed that the highest shoot number (8.425 shoots/jar) was significantly observed in the Hermes cultivar (Fig.1), followed by the Spunta cultivar (7.524 shoots/jar). Lower values were recorded for the other cultivars. The LR cultivar recorded the lowest shoot number. Data of the main effect of treatment revealed that both treatments contained 0.5mg/L or 0.25mg/L KIN significantly showed a similar values of shoot number (8.552, 8.500 shoots/Jar, respectively) which significantly surpassed all other treatments. Regarding the interaction, similar shoot numbers were recorded for the Hermes cultivar cultured on media containing 0.25 mg/L KIN alone, 0.5 mg/L KIN alone, or 0.25 mg/L NAA alone. However, the treatment of Spunta cultured on medium containing 0.5 mg/L NAA only showed a significant similar result followed by Cara cultivar cultured on 1.0 mg/L KIN alone. Lower sporadic values were observed with the other treatments.

In Table (5), results of the main effect of cultivars observed that the longest shoots were significantly observed in the Spunta and Hermes cultivars (7.044, 6.908 cm/shoot,

respectively). Lower values were observed with other cultivars, with LR significantly showing the shortest shoots at 4.927 cm/shoot. Data on the main effect of treatments revealed that the combination of 0.25 mg/L KIN + 1.0 mg/L NAA significantly resulted in the longest shoots (7.000 cm), surpassing most other treatments. However, three other treatments (0.5 mg/L KIN + 1.0 mg/L NAA), (1.0 mg/L KIN + 1.0 mg/L NAA), and (1.0 mg/L NAA alone) were not significantly different from this highest value with (6.892, 6.720 and 6.664 cm/shoot, respectively), indicating comparable effectiveness among these specific growth regulator combinations. In contrast, treatment with 1.0 mg/L KIN alone showed stunted shoots with a mean length of only (5.006 cm/shoot). As for the interaction, Six treatments showed significant results, but no significant differences were found between them. Three of these treatments involved Spunta explants (9.3, 8.14 and 8.29 cm/shoot ) cultured on medium contained 0.25 mg/L KIN+0.5 mg/L NAA, 0.25 mg/L KIN only and the control medium, respectively, and the other three treatments were with Hermes explants (9.24, 8.81 and 8.24 cm/shoot) cultured on medium contained 0.25mg/L KIN + 1.0mg/L NAA, 1.0mg/L NAA only and 1.0mg/L KIN + 1.0 mg/L NAA, respectively. Significantly lower sporadic values were observed with other interactions.

In Table (6), results of the main effect of cultivars observed that the highest average number of leaves 36.81 leaves/jar was significantly recorded with Hermes cultivar, followed by other cultivars, with Cara significantly showing the lowest leaf number with (27.38 leaves/jar). Significant differences were observed among the varieties for this trait. Data on the main effect of treatments revealed that the medium contained (0.5 and 0.25 mg/L KIN alone) both were significantly produced the highest leaf yield, averaging 38.26 and 37.92 leaves, respectively across all cultivars. In contrast, the treatment with 1.0 mg/L NAA

resulted in the lowest significant leaf production, averaging just (25.2 leaves/jar), emphasizing the importance of cytokinins in leaf development. As for the interaction, significantly similar values of leaf numbers were observed with Hermes cultivated on media containing 0.25 mg/L or 0.5 mg/L KIN alone, both reaching a peak of (53.3 leaves/jar), the maximum overall. Cara, on the other hand, recorded the lowest leaf production of 17.2 leaves/Jar on 0.25 mg/L KIN + 0.25 mg/L NAA, demonstrating the genotype specific responses and significant interactions between varieties and treatments. Other treatments exhibited inconsistent and comparatively lower leaf yields, highlighting the variability and limited effectiveness of those specific growth regulator combinations.

### 3.3. Rooting stage

In Table (7) results of the main effect of cultivars observed that the highest number of roots were not significantly recorded by Spunta, Cara and Hermes with (39.72, 34.19 and 32.42 roots/Jar, respectively) Lower values were observed with other cultivars, with Silana and LR significantly showed the smallest root number at 22.36 and 25.69 roots/jar, respectively. Significant differences were observed among the varieties for this trait. Data on the main effect of treatments revealed that the medium containing 0.5 mg/L NAA + 0.1 mg/L KIN significantly produced the highest root yield, averaging 56 roots/Jar across all Treatments. In contrast, the treatment with 2.0 mg/L NAA + 2.0 mg/L KIN resulted in significantly the lowest root production, averaging just 14.34 roots/Jar. Regarding the interaction, three treatments showed significantly higher root numbers, without significant differences between them. Explants of the Cara, Hermes, and Spunta cultivars were cultured on a medium containing 0.5 mg/L NAA + 1.0 mg/L KIN and 1.0 mg/L KIN only, with average root numbers of (48.0, 60.67, and 57.67 roots/jar, respectively).

In Table (8) results of the main effect of cultivars revealed that the highest average root length (6.514) cm was significantly recorded for Spunta cultivar, followed by Silana (4.944 cm). Lower values were observed with other cultivars, with Cara displayed the shortest root length at 3.903 cm. Data on the main effect of treatments revealed that the medium containing 0.5 mg/L NAA significantly produced the longest roots, averaging 6.78 cm across all cultivars. However, other high performing treatments, including 0.1 mg/L KIN alone (5.613 cm), 0.5 mg/L KIN alone (6.047 cm), and 0.5 mg/L NAA + 0.5 mg/L KIN (5.52 cm), did not differ significantly. In contrast, the treatment with 2.0 mg/L NAA alone produced the shortest root length, averaging just 3.147 cm. As for the effect of the interaction, approximately eight treatments that produced the highest root lengths, with no significant differences among them. These treatments included combinations such as 1.0 mg/L NAA + 0.5 mg/L KIN, producing 9.667 cm in Spunta explants, 2.0 mg/L NAA + 0.5 mg/L KIN, producing 7.66 cm and 6.66 cm in Spunta and Silana explants, respectively, and 0.5 mg/L alone, producing 7.16 cm and 6.56 cm in Cara and Hermes explants, respectively. While these treatments were statistically similar to each other, they were significantly superior to the other treatments. Other treatments exhibited inconsistent and comparatively lower root lengths, highlighting the variability and limited effectiveness of these specific growth regulator combinations.

#### 3.4. Acclimatization stage

The acclimatization stage revealed significant differences among the five potato cultivars in various growth parameters. Survival rate varied significantly among cultivars. Lady Rosetta and Spunta showed the highest survival rates at 96%, demonstrating superior adaptability during the acclimatization process. Silana and Hermes followed closely with a 92% survival rate. Cara exhibited the lowest survival rate at 88%, indicating it may

be more sensitive to the transition from *in vitro* to *ex vitro* conditions. Shoot length also differed significantly between cultivars. Hermes displayed the most vigorous shoot growth (Fig.3), with an average length of 20 cm, significantly outperforming all other cultivars. Cara, Spunta, Silana, and Lady Rosetta showed comparable shoot lengths, ranging from 13.8 to 16 cm, with no significant differences among them.

The number of leaves per plant did not show statistically significant differences among the cultivars. All cultivars produced between 9.2 and 11.6 leaves per plant, suggesting similar leaf development rates across the different genetic backgrounds. Similarly, the number of roots did not differ significantly among cultivars. Root numbers ranged from 8.2 to 13.4 per plant, with Hermes showing a tendency towards higher root production, although not statistically different from the others. Root length showed some variability among cultivars. Hermes produced the longest roots with an average of 9.6 cm, significantly higher than Cara and Lady Rosetta, which had the shortest roots at 5 cm and 5.4 cm respectively. Spunta and Silana showed intermediate root lengths, not significantly different from either the highest or lowest groups.

#### 3.5. Minitubers production

The results of the minituber production experiment were analyzed in two distinct parts: the vegetative growth over the 8 week production period and the characteristics of the tubers formed. During the vegetative phase, we monitored key parameters such as Surviving, number of shoots, shoot length, number of nodes and number of leaves to assess the overall plant development. Following this, we examined the tubers produced, focusing on metrics including tuber number per plant, average tuber weight and the volume of tubers. This two-pronged approach allowed us to comprehensively evaluate both the above-ground growth and the below-ground tuber

formation, providing insights into the efficacy of our production methods.

In Table (10) the experiment revealed significant differences in vegetative growth among the four potato cultivars. Hermes demonstrated superior performance in most vegetative parameters. It had the highest survival rate (93.33%), significantly outperforming other cultivars. Hermes also produced the most shoots per plant (5.667) and had the highest number of nodes (40.33) and leaves (51.33). Lady Rosetta showed the second-best overall vegetative growth, with the longest shoot length (60.23 cm) and the second-highest numbers of nodes (26.67) and leaves (32.67). Its survival rate (53.33%) was significantly higher than Cara and Spunta, but lower than Hermes. Cara exhibited moderate performance, with values often falling between Lady Rosetta and Spunta. Spunta consistently showed the poorest vegetative growth, with the lowest survival rate (20%), fewest shoots (1), shortest shoot length (29.33 cm), and lowest numbers of nodes (8.667) and leaves (10.67).

In Table (11) the tuber production phase revealed a different pattern of performance among the cultivars. Lady Rosetta excelled with a 100% survival rate, significantly higher than all other cultivars. It produced a high number of tubers (7.6 per plant) and had the highest tuber weight (40.2 g) and volume (38.6 cm<sup>3</sup>). Hermes and Spunta both achieved 80% survival rates, significantly higher than Cara but lower than Lady Rosetta. However, their tuber production differed markedly. Hermes nearly matched Lady Rosetta in tuber weight (41.56 g) and volume (36.2 cm<sup>3</sup>), with a slightly lower but not significantly different number of tubers (6.8). In contrast, Spunta produced the fewest tubers (2.2) with the lowest weight (5.12 g) and volume (5.8 cm<sup>3</sup>). Cara showed the lowest survival rate in tuber production (60%) but surprisingly produced the highest number of tubers (10.2). However, these tubers had moderate weight (21.6 g) and volume (21.8

cm<sup>3</sup>), significantly lower than Lady Rosetta and Hermes but higher than Spunta (Fig.4).

Interestingly, the cultivars performance in vegetative growth did not always directly correlate with their tuber production. Hermes exhibited strong performance in both phases. Lady Rosetta showed average vegetative growth but excelled in tuber production and survival. Spunta, despite poor vegetative growth, achieved a higher survival rate in tuber production but still produced the fewest and smallest tubers. Cara's performance was inconsistent, with poor vegetative survival but the highest tuber count, albeit with moderate size. These results highlight the complex relationship between vegetative growth and tuber production in different potato cultivars. They suggest that optimal cultivar selection may depend on specific production goals, with some cultivars favoring vegetative growth and others excelling in tuber formation and survival.

### 3.6. Super elite production

The production of super elite potato tubers from four cultivars (Cara, Spunta, Lady Rosetta, and Hermes) yielded significant insights into their performance characteristics, underscoring the critical role of cultivar selection in seed potato programs (Fig.5). In Table 12, Tuber number per plant showed no statistically significant differences among the cultivars, as indicated by the identical 'A' designation and an LSD of 2.222. Hermes produced the highest mean number of tubers (10.7), while Lady Rosetta had the lowest (8.9), but these differences were not statistically significant. However, substantial variations were observed in tuber weight and volume, highlighting the importance of these parameters in super elite seed production. Cara demonstrated superior performance in both weight (907.5g) and volume (845.5cm<sup>3</sup>), significantly outperforming all other cultivars. Spunta ranked second in both metrics (646.4g and 605cm<sup>3</sup>), significantly different from both Cara and the remaining cultivars. Lady Rosetta

and Hermes formed a statistically indistinguishable group with the lowest weights (407g and 480.8g respectively) and volumes (391cm<sup>3</sup> and 469cm<sup>3</sup>).

The results for minituber and super elite production encompass four of the five initially micro propagated cultivars: Spunta, Cara, Lady Rosetta, and Hermes. The Silana cultivar did not produce sufficient plant material during the acclimatization stage to proceed to these later phases of the study. This unexpected limitation in Silana's performance during early stages highlights the variability in cultivar response to *in vitro* propagation and acclimatization conditions, an important consideration in potato seed production systems.

#### **4. DISCUSSION**

This study focused on the micropropagation of five potato cultivars ( Cara, Silana, Hermes, Spunta and Lady Rosetta). The protocol involved sterilization with 1% sodium hypochlorite, followed by shoot multiplication and rooting using various concentrations and combinations of kinetin and NAA growth regulators. Well-rooted plantlets were successfully acclimatized in a 2:1 mixture of peatmoss and sand then these plantlets were used to produce minituber and super elite.

The sterilization of explants is a critical step in micropropagation protocols, particularly for potato cultivars. In this study, we employed a surface sterilization method using 1% sodium hypochlorite, which aligns with established practices in the field. This approach balances efficacy in decontamination with minimal damage to the plant tissue. Our methodology is consistent with several studies in the literature. For instance, Badoni and Chauhan, (2010) explored the use of 1% sodium hypochlorite for potato sprout sterilization, comparing it with mercuric chloride at various exposure times. Similarly, Westcott *et al.*, (1977) and Goodwin, (1981) successfully utilized 1% aqueous sodium hypochlorite for sterilizing single node cuttings across multiple potato cultivars, demonstrating the broad applicability of this

method. Ebad *et al.*, (2015) found that 20% Clorox (sodium hypochlorite) applied for 20 minute yielded optimal results, balancing high survival rates with low contamination. Our choice of 1% sodium hypochlorite strikes a balance between efficacy and tissue preservation, which is particularly important when working with multiple cultivars that may have varying sensitivities to sterilizing agents.

While our protocol employed a straightforward approach, it's worth noting that other researchers have investigated more complex sterilization procedures. Bošnjak Mihovilović *et al.*, (2024) Found that a 4% NaClO solution effectively eliminated these pathogens without adversely affecting seed germination. Hoque, (2010) explored the use of mercuric chloride in their sterilization protocols, which can be highly effective but requires careful handling due to its toxicity.

Building upon the successful surface sterilization of explants. Our study focused on optimizing shoot multiplication and root induction through the strategic application of plant growth regulators, specifically kinetin (KIN) and 1-Naphthaleneacetic acid (NAA). The data indicates significant genotypic variation among the cultivars. The interplay between cytokinins and auxins is crucial in regulating organogenesis in potato micropropagation. Our findings align with the established understanding that cytokinins, such as kinetin, play a critical role in shoot proliferation by promoting axillary shoot development and reducing apical dominance (George *et al.*, 2008). Kinetin suppresses the conversion of indoleacetic acid (IAA) into inactive conjugates, thereby increasing the availability of free IAA, which is essential for promoting growth and branching (Lau and Yang, 1973). By inhibiting IAA oxidation, kinetin helps maintain higher levels of active auxin, facilitating shoot multiplication and branching (Duszka *et al.*, 2009). In studies of woody plants, auxin was shown to inhibit bud outgrowth, while kinetin's modulation of auxin



levels can promote branching by alleviating this inhibition (Yang *et al.*, 2022).

We found that low concentrations of kinetin, when balanced with NAA, can support both shoot multiplication and subsequent root induction. This observation is consistent with reports by Nugroho *et al.*, (2024), who noted that optimizing kinetin concentrations alongside NAA not only supported shoot formation but also facilitated subsequent rooting processes. Rout *et al.*, (2023) found that KIN, when used at 0.25 mg/L, resulted in the highest shoot bud initiation, yielding approximately 3.5 shoots per explant in a short duration.

The inclusion of NAA in our protocol is supported by George *et al.*, (2008), who emphasized the beneficial effects of low auxin concentrations in conjunction with cytokinins during the multiplication stage. Our approach of using a combination of kinetin and NAA aligns with the findings of Badoni and Chauhan, (2009), who reported that low concentrations of NAA (0.1 mg/l) combined with moderate concentrations of kinetin (0.01 mg/l) resulted in good development of complete plantlets from potato meristem tips.

The effectiveness of our protocol in promoting both shoot multiplication and root induction in a single medium formulation is particularly noteworthy. This approach streamlines the micropropagation process, potentially reducing the time and resources required for plantlet production. Our results are in line with Ebad *et al.*, (2015), who found that adding 0.2 mg/l NAA to the medium adjusted the auxin-cytokinin ratio to a point where normal plantlets with both shoots and roots were produced in a single step. Furthermore, our use of nodal segments containing axillary buds as explants is supported by multiple studies in the literature. (Rai *et al.*, 2012) demonstrated the efficiency of using single node cuttings for mass multiplication of potato microplants. This approach activates axillary buds, a method favored in early studies by

Westcott *et al.*, (1977). Our optimized protocol for shoot multiplication and root induction in potato micropropagation leverages the synergistic effects of kinetin and NAA. By carefully balancing these growth regulators, we have developed a robust system that promotes efficient organogenesis, contributing to the advancement of potato micropropagation techniques. Future studies could further refine this approach by exploring cultivar specific responses to growth regulator combinations, potentially enhancing the efficiency and applicability of this protocol across diverse potato genotypes.

During the acclimatization stage, Hermes demonstrated superior performance in shoot and root growth, while Lady Rosetta and Spunta excelled in survival rate. Cara generally showed lower performance across most parameters, suggesting it may require more careful management during acclimatization. Our acclimatization results align with the findings of Ibrahim *et al.*, (2016), demonstrating a survival rate exceeding 80% after four weeks. The successfully acclimatized potato plants were subsequently transferred to 25 cm diameter plastic pots to promote further growth and facilitate minituber formation, where they exhibited robust development under greenhouse conditions. The lack of significant differences in leaf and root numbers indicates that these traits may be more stable across cultivars during the acclimatization phase. The number of tubers per plant is a critical parameter in minituber production for seed purposes (Ahloowalia, 1994). Our results showed a range of 2.2 to 10.2 tubers per plant across the studied cultivars. This variability is consistent with observations in the literature, although our range extends beyond that typically reported.

Struik, (2007) stated that minituber production usually falls within the range of 2-5 tubers per planted plant. While our results for Spunta (2.2 tubers/plant) align with this range, Cara (10.2 tubers/plant) and Lady Rosetta (7.6

tubers/plant) notably exceeded it. This suggests these cultivars may have higher potential for minituber production under our experimental conditions. Our findings for Spunta closely align with the lower end of the range reported by Grigoriadou and Leventakis, (1999), who observed 1.85-2.52 tubers per plant. In contrast, the higher yields observed for Cara and Lady Rosetta are more comparable to the findings of (Corrêa *et al.*, 2008), who reported average yields of 7.00-8.31 minitubers per plant. Importantly, our results for all cultivars, except Spunta, surpassed the very low average of 0.26-3.07 tubers per plant reported by (Ahloowalia, 1994). This discrepancy could be attributed to differences in growing conditions, cultivation techniques, or genetic improvements in the cultivars over time. The cultivar dependent variation in tuber number per plant observed in our study corroborates findings from multiple researchers (Ahloowalia, 1994; Struik, 2007). This reinforces the importance of cultivar selection in minituber production systems. Our minitubers results are align with Struik and Wiersema, (2023) who found that minitubers may vary in size from 5–25 mm, although in numerous potato seed production systems, larger minitubers are frequently observed. Studies show that specific potato cultivars, such as Nicola, exhibit superior performance in terms of shoot multiplication and minituber production under greenhouse conditions. Greenhouse conditions allow for better control over environmental factors, resulting in higher yields and healthier plants (Etdzaeva and Oves, 2022).

In the super elite production, according to (Toma, 2022), while the greenhouse method is widely supported, some studies suggest that open-field conditions may yield larger tubers, indicating a potential trade-off between quantity and size of the harvest. These results emphasize several key aspects of super elite seed production: Cultivar-specific performance: The significant variations in weight and volume despite similar tuber

numbers underscore the importance of cultivar selection in seed potato programs. This variability can impact the efficiency of seed production and the subsequent performance of progeny tubers. Quality metrics: While tuber number is important, the substantial differences in weight and volume suggest that these parameters may be more discriminating indicators of super elite seed quality and potential yield in subsequent generations. Production efficiency: Cultivars producing larger tubers (like Cara) may offer advantages in terms of storage, handling, and potentially in the vigor of seed pieces cut from these tubers. Genetic diversity: The range of performance across cultivars highlights the genetic diversity available in potato breeding programs and the potential for selecting cultivars optimized for specific production goals or environmental conditions. Economic implications: The significant differences in tuber weight and volume among cultivars could have substantial economic impacts on seed potato production, influencing factors such as storage requirements, transportation costs, and the number of potential seed pieces per tuber.

These findings underscore the complexity and importance of cultivar evaluation in super elite seed potato production. They provide valuable data for optimizing seed potato programs, potentially improving the efficiency and quality of subsequent generations of seed and commercial potato production. Furthermore, this research contributes to our understanding of cultivar specific traits in high grade seed production, which is crucial for maintaining the health, vigor, and genetic integrity of potato planting material in commercial agriculture.

## 5. CONCLUSION

This study on the micropropagation of five potato (*Solanum tuberosum* L.) cultivars (Hermes, Spunta, Lady Rosetta, Cara, and Silana) has yielded several significant findings with important implications for potato tissue culture and propagation. The research clearly

demonstrated that different potato cultivars exhibit varying responses to *in vitro* culture conditions and growth regulator treatments, underscoring the importance of optimizing micropropagation protocols for each specific cultivar to maximize efficiency.

The study identified effective concentrations of kinetin (KIN) and Naphthalene Acetic Acid (NAA) for various stages of micropropagation. For shoot multiplication, the combination of 0.5 mg/L KIN + 1.0 mg/L NAA produced the highest number of nodes (21.28) across cultivars. In the rooting stage, 1.0 mg/L KIN + 0.25 mg/L NAA resulted in the highest rooting percentage (97.66%). These findings provide valuable guidance for optimizing growth regulator combinations in potato tissue culture. Among the cultivars studied, Hermes showed the highest survival percentage (99.6%) and shoot number (8.425) during multiplication, Spunta produced the longest shoots (7.044 cm) and the highest average number of roots (39.72) during the rooting stage, demonstrating its superior rooting ability compared to other cultivars. During the acclimatization phase, Lady Rosetta and Spunta demonstrated superior performance with 96% survival rates. The study achieved high survival rates (88-96%) during acclimatization across all cultivars, indicating the effectiveness of the developed protocols for producing viable *ex vitro* plants.

These findings have important implications for large-scale, disease-free seed potato production in Egypt. By optimizing cultivar-specific micropropagation protocols, it is possible to significantly enhance the efficiency and output of potato tissue culture systems. This could contribute to reducing Egypt's dependence on imported seed potatoes and potentially increase the country's capacity for potato production and export.

Future research directions could include fine-tuning growth regulator concentrations for each cultivar to further optimize shoot multiplication and rooting. Investigating the

effects of alternative cytokinins and auxins on micropropagation efficiency could uncover additional strategies for improving growth outcomes. Exploring the use of advanced systems, such as temporary immersion systems or bioreactors, for scaling up production would also be a valuable area of study, particularly for commercial applications.

Furthermore, conducting field trials to compare the agronomic performance of micropropagated plants with conventionally propagated ones would provide critical insights into their practical utility. Investigating the genetic stability of micropropagated plants across multiple subcultures could help ensure the reliability of these protocols for long-term commercial use. Together, these studies would advance the scalability, reliability, and practical application of micropropagation techniques in potato production systems.

## 6. REFERENCES

- Ahloowalia, B.S. (1994) 'Production and performance of potato mini-tubers', *Euphytica*, 75(3), pp. 163–172. Available at: <https://link.springer.com/article/10.1007/bf00025600>
- Ahloowalia, B.S. (1999) 'Production of min-seed tubers using a modular system of plant micropropagation', *Potato Research*, 42(3–4), pp. 569–575. Available at: <https://link.springer.com/article/10.1007/BF02358173>.
- Badoni, A. and Chauhan, J.S. (2009) 'Effect of growth regulators on meristem tip development and *in vitro* multiplication of potato cultivar 'Kufri Himalini'', *Indian Journal of Crop Science*, 4(1–2), pp. 155–157.
- Badoni, A. and Chauhan, J.S. (2010) 'Conventional vis-a-vis biotechnological methods of propagation in potato: A review', *Stem Cell*, 1(1), pp. 1–6.
- Bošnjak Mihovilović, A., Kereša, S., Lazarević, B., Topolovec Pintarić, S., Martinko, K., Marković, Z., Turkalj, K. and Habuš Jerčić, I. (2024) 'The use of sodium

- hypochlorite and plant preservative mixture significantly reduces seed-borne pathogen contamination when establishing *in vitro* cultures of wheat (*Triticum aestivum* L.) seeds', *Agriculture*, 14(4), p. 556. Available at: <https://doi.org/10.3390/agriculture14040556>.
- Corrêa, R.M., Pinto, J.E.B.P., Pinto, C.A.B.P., Faquin, V., Reis, É.S., Monteiro, A.B. and Dyer, W.E. (2008) 'A comparison of potato seed tuber yields in beds, pots and hydroponic systems', *Scientia Horticulturae*, 116(1), pp. 17–20.
- Duszka, K., Clark, B.F., Massino, F. and Barciszewski, J. (2009) 'Biological activities of kinetin', in Ramawat, K.G. (ed.) *Herbal drugs: ethnomedicine to modern medicine*. Berlin, Heidelberg: Springer, pp. 369–380. Available at: [https://doi.org/10.1007/978-3-540-79116-4\\_20](https://doi.org/10.1007/978-3-540-79116-4_20).
- Ebad, F.A.-S., El-Sebai, M., and El-Kazzaz, A.A. (2015) 'Micropropagation of four potato cultivars *in vitro*', *Academia Journal of Agricultural Research*, 3(9), pp. 184–188. DOI: 10.15413/ajar.2015.0145.
- Etdzaeva, K.T. and Oves, E.V. (2022) 'Production of mini potato tubers in two rotations of protected ground in the conditions of North Ossetia-Alania', *Agricultural Science Euro-North-East*, 23(4), pp. 441–449. Available at: <https://doi.org/10.30766/2072-9081.2022.23.4.441-449>.
- Freed, R., Eisensmith, S.P., Goetz, S., Reicosky, D., Smail, V.W. and Wolberg, P. (1991) *MSTAT-C: A software program for the design, management, and analysis of agronomic research experiments*. Version 2.1. East Lansing, MI: Michigan State University, Department of Crop and Soil Sciences.
- George, E.F., Hall, M.A., and De Klerk, G.J. (2008) *Plant propagation by tissue culture*. 3rd edn. The Netherlands: Springer, pp. 65–175.
- Gisyuk, A.A., Volkov, D.I. and Shchegorets, O.V. (2023) 'Multiplying *in vitro* plantlets of virus-free pre-basic potato under greenhouse conditions', *Vegetable crops of Russia*, (1), pp. 23–29. Available at: <https://doi.org/10.18619/2072-9146-2023-1-23-29>.
- Goodwin, P. (1981) 'Rapid propagation of potato by single node cuttings', *Field Crops Research*, 4, pp. 165–173.
- Grigoriadou, K. and Leventakis, N. (1999) 'Large scale commercial production of potato minitubers, using *in vitro* techniques', *Potato Research*, 42, pp. 607–610.
- Hoque, M.E. (2010) '*In vitro* tuberization in potato (*Solanum tuberosum* L.)', *Plant Omics*, 3(1), pp. 7–11.
- Ibrahim, A.I., Emara, H.A., Nower, A.A., and Abodiab, A.Y. (2016) '*In vitro* cultivation of potato plants', *International Journal of Current Microbiology and Applied Sciences*, 5(12), pp. 858–868. Available at: <http://dx.doi.org/10.20546/ijemas.2016.512.094>
- Kim, H.H., Yoon, J.W., Park, Y.E., Cho, E.G., Sohn, J.K., Kim, T.S. and Engelmann, F. (2006) 'Cryopreservation of potato cultivated varieties and wild species: critical factors in droplet vitrification', *CryoLetters*, 27(4), pp. 223–234.
- Lau, O.-L. and Yang, S.F. (1973) 'Mechanism of a Synergistic Effect of Kinetin on Auxin-induced Ethylene Production: Suppression of Auxin Conjugation', *Plant Physiology*, 51(6), pp. 1011–1014. Available at: <https://doi.org/10.1104/pp.51.6.1011>.
- Misal, V.D. and Chavan, A.M., 2024. '*In vitro* micropropagation and mass multiplication of *Stevia rebaudiana* Bertoni', *World Journal of Advanced Research and Reviews*, 23(1), pp. 250–254. Available at: <https://doi.org/10.30574/wjarr.2024.23.1.2027>.
- Murashige, T. and Skoog, F. (1962) 'A revised medium for rapid growth and bioassays with

- tobacco tissue cultures', *Physiologia Plantarum*, 15(3), pp. 473-497.
- Nugroho, K., Kosmiatin, M., Santoso, T.J., Sukma, D., Purwito, A., Husni, A. and Martasari, C., (2024). Optimization of kinetin concentrations and medium compositions for citrus shoot multiplication from cotyledonary node. *Biotropia*, 31(1). Available at: <https://doi.org/10.11598/btb.2024.31.1.2136>.
- Observatory of Economic Complexity (2022) *Egypt (EGY) and Potatoes*. Available at: <https://oec.world/en/profile/bilateral-product/potatoes/reporter/egy>.
- Rai, R., Diengdoh, L.C., Srivastava, A.K., and Bag, T.K. (2012) 'Efficiency of different nodal segments for potato micropropagation', *Environment and Ecology*, 30(3), pp. 594-597.
- Rout, B.M., Bhatia, A.K., Bora, L., Chauhan, S.S.A., Sapra, L., and Singh, T. (2023) 'Optimization of an efficient micropropagation protocol and the assessment of genetic fidelity of *Solanum tuberosum* L. cv Kufri Neelkanth', *Environment and Ecology*.
- Sakara, H. and Badour, A. (2020) 'Bio-fortification of potato plants with different nitrogen rates and applied iron forms in presence and absence of *Azotobacter* sp.', *Egyptian Journal of Soil Science*, 60(4), pp. 449-467.
- Sangar, R.B.S., Agrawal, H.O. and Nagaich, B.B. (1993) 'Studies on the translocation of potato viruses X and Y in potatoes', *Indian Phytopathology*, 46(2), pp. 110-117. Available at: <https://www.cabidigitallibrary.org/doi/full/10.5555/19942303857>
- Särekanno, M. (2011) 'Analysis of productivity indicators of field-grown potato meristem plants multiplied by different methods', PhD thesis, Estonian University of Life Sciences. Available at: <https://dspace.emu.ee/handle/10492/153>.
- Singh, V., Deen, B. and Singh, S. (2023) 'Micropropagation of minor fruit crops of India: a review', *Agricultural Reviews*, 44(2), pp. 259-263. Available at: <https://doi.org/10.18805/ag.R-2569>.
- Smirnova, Y. and Podolian, E. (2024) 'Techniques for increasing the efficiency of microclonal propagation of potatoes (review)', *Agricultural Science Euro-North-East*, 25, pp. 319-329. Available at: <https://doi.org/10.30766/2072-9081.2024.25.3.319-329>.
- Struik, P.C. (2007) 'The Canon of Potato Science: 25. Minitubers', *Potato Research*, 50(3-4), pp. 305-308. Available at: <https://doi.org/10.1007/s11540-008-9051-z>.
- Struik, P.C. and Wiersema, S.G. (2023) *Seed potato technology*, Leiden, The Netherlands: Wageningen Academic. Available From: Brill <https://doi.org/10.3920/978-90-8686-759-2>
- Tolessa, E.S., Belew, D. and Debela, A. (2017) 'Effect of nitrogen rates and irrigation regimes on nitrogen use efficiency of potato (*Solanum tuberosum* L.) in southwest Ethiopia', *Archives of Agriculture and Environmental Science*, 2(3), pp. 170-175. Available at: <https://journals.aesacademy.org/index.php/aaes/article/view/229>.
- Toma, R.S. (2022) 'minitubers production of four potato (*Solanum tuberosum* L.) cultivars by tissue culture technique', *iraqi journal of agricultural sciences*, 53(5), pp. 1058-1066. Available at: <https://doi.org/10.36103/ijas.v53i5.1619>.
- U.S. Department of Agriculture (USDA) 2018, *Egypt's Import Supply of European-Origin Seed Potatoes Challenged*, Cairo, Egypt, 26 December.
- Wang, P. and Hu, C. (1982) 'In vitro mass tuberization and virus-free seed-potato production in Taiwan', *American Potato Journal*, 59(1), pp. 33-37. Available at: <https://doi.org/10.1007/BF02854881>.

Wang, Q.C. and Valkonen, J.P.T. (2008) ‘Elimination of two viruses which interact synergistically from sweetpotato by shoot tip culture and cryotherapy’, *Journal of Virological Methods*, 154(1–2), pp. 135–145. Available at: <https://doi.org/10.1016/j.jviromet.2008.08.006>.

Westcott, R.J., Henshaw, G.G. and Roca, W.M. (1977) ‘Tissue culture storage of potato

germplasm: Culture initiation and plant regeneration’, *Plant science letters*, 9(4), pp. 309–315.

Yang, L., Zhu, S. and Xu, J. (2022) ‘Roles of auxin in the inhibition of shoot branching in “Dugan” fir’, *Tree Physiology*. Edited by I. Allona, 42(7), pp. 1411–1431. Available at: <https://doi.org/10.1093/treephys/tpac008>.

**Table (1):** Evaluating the effect of different growth regulators (Kin, NAA) with different concentrations (0.0, 0.25, 0.5 and 1.0 mg/l) on the shoot multiplication of potato cultivars.

NAA	KIN	0.0 (mg/l)	0.25 (mg/l)	0.5 (mg/l)	1.0 (mg/l)
0.0 (mg/l)		T1	T2	T3	T4
0.25 (mg/l)		T5	T6	T7	T8
0.5 (mg/l)		T9	T10	T11	T12
1.0 (mg/l)		T13	T14	T15	T16

**Table (2):** Evaluating the effect of different growth regulators KIN (0.0, 0.1 and 0.5) and NAA (0.0, 0.5, 1.0, and 2.0) mg/l and the interaction between them on the potato cultivars roots induction.

KIN	NAA	0.0 (mg/l)	0.5 (mg/l)	1.0 (mg/l)	2.0 (mg/l)
0.0 (mg/l)		T1	T2	T3	T4
0.1 (mg/l)		T5	T6	T7	T8
0.5 (mg/l)		T9	T10	T11	T12

**Table (3):** Effect of KIN and NAA levels on survival rate (%) of micropropagated potato cultivars.

Treatment		Cultivars					Treatment mean
KIN (mg/l)	NAA (mg/l)	Cara	Spunta	Silana	LR	Hermes	
0.0	0.0	83a-i	69.6h-l	96.6ab	93.2a-e	96.6ab	<b>87.80ab</b>
0.25		83a-i	89.8a-g	100a	66.5i-m	100a	<b>87.86ab</b>
0.5		93.2a-e	73g-k	100a	91.5a-f	100a	<b>91.54ab</b>
1.0		93.3a-e	86.6a-h	100a	75.7f-k	100a	<b>91.12ab</b>
0.0	0.25	100a	87.38a-g	93.2a-e	77.78d-j	100a	<b>91.67ab</b>
0.25		59.9klm	100a	76.3e-k	89a-g	100a	<b>85.04bc</b>
0.5		100a	100a	89.9a-g	79.8b-i	100a	<b>93.94a</b>
1.0		93.3a-e	83.1a-i	96.6ab	87.3a-g	100a	<b>92.06ab</b>
0.0	0.5	83.3a-i	100a	93.3abcde	83a-i	100a	<b>91.92ab</b>
0.25		100a	60.68jklm	79.8b-i	50.5m	100a	<b>78.20c</b>
0.5		100a	87.26a-g	95.13abc	93.1a-e	100a	<b>95.10a</b>
1.0		90a-g	78.76c-i	100a	90.2ab-g	100a	<b>91.79ab</b>
0.0	1.0	89.9a-g	66.5i-m	79.7b-i	91.6a-f	100a	<b>85.54bc</b>
0.25		89.8a-g	73g-k	96.6ab	94.2abcd	96.6ab	<b>90.04ab</b>
0.5		86.5a-h	53.1lm	96.6ab	94.2abcd	100a	<b>86.08b</b>
1.0		93.3a-e	51.56m	85.11a-h	93.2a-e	100a	<b>84.63bc</b>
<b>Cultivar mean</b>		<b>89.91b</b>	<b>78.77d</b>	<b>92.43b</b>	<b>84.42c</b>	<b>99.57a</b>	

**Table (4):** Influence of KIN and NAA concentrations on shoot number per jar in micropropagated potato cultivars.

Treatment		Cultivars					Treatment mean
KIN (mg/l)	NAA (mg/l)	Cara	Spunta	Silana	LR	Hermes	
0.0	0.0	6n-w	7.3i-q	6.28m-u	7.8g-o	8.2f-n	<b>7.116bc</b>
0.25		7.6h-p	9.7c-h	7.6h-p	4u-\	13.6a	<b>8.500a</b>
0.5		8.8e-k	7.3i-q	6.2n-v	7.76g-o	12.7ab	<b>8.552a</b>
1.0		10.8bcde	4.6r-\	5.8o-x	3.82w-\	11.6abc	<b>7.324b</b>
0.0	0.25	5.2q-[	6.38l-t	4.6r-\	4.78r-\	10.1c-g	<b>6.212cd</b>
0.25		2.9[\	9.4c-i	4.8r-\	6n-w	10.2cdef	<b>6.660bcd</b>
0.5		6.4l-t	8.8e-k	5.5o-y	4.7r-\	8.8e-k	<b>6.840bc</b>
1.0		5.1q-[	8.7e-l	5.1q-[	5.2q-[	9.1de-j	<b>6.640bcd</b>
0.0	0.5	3.6xyz[\	11.3abcd	3.4yz[\	5.3p-z	11.3abcd	<b>6.980bc</b>
0.25		4.5s-\	6.1n-w	3.9v-\	6n-w	8.3f-n	<b>5.760de</b>
0.5		7.3i-q	8.2f-n	6.13n-w	6.8j-s	7.3i-q	<b>7.146bc</b>
1.0		3.5xyz[\	8.6e-m	5.5o-y	5.3p-z	7.8g-o	<b>6.140cd</b>
0.0	1.0	3.6xyz[\	6.7k-s	3.3yz[\	5.2q-[	5.2q-[	<b>4.800e</b>
0.25		2.7\	6.9j-r	4.7r-\	10cdefg	3.8w-\	<b>5.620de</b>
0.5		3.1z[\	5.2q-[	4.3t-\	8.2f-n	3.9v-\	<b>4.940e</b>
1.0		4u-\	5.2q-[	5.56o-y	6.3m-u	2.9[\	<b>4.792e</b>
<b>Cultivar mean</b>		<b>5.319d</b>	<b>7.524b</b>	<b>5.167d</b>	<b>6.073c</b>	<b>8.425a</b>	

“Means followed by the same letter in the same column are not significantly different according to Fisher’s LSD test at P ≤ 0.05. Symbols such as n-w, i-q, etc., indicate statistical groupings based on the test results.”

**Table (5):** Impact of KIN and NAA concentrations on shoot length (cm/shoot) of micropropagated potato cultivars.

Treatment		Cultivars					Treatment mean
KIN (mg/l)	NAA (mg/l)	Cara	Spunta	Silana	LR	Hermes	
0.0	0.0	6.35g-t	8.29a-e	4.43v-]	5.62k-z	6.25h-u	<b>6.188bcd</b>
0.25		5.75k-w	8.14a-f	4.85s-\]	3.77\]	5.96j-v	<b>5.694defg</b>
0.5		5.38n-]	7.02d-m	4.85s-\]	4.46v-]	5.26p-\]	<b>5.394fg</b>
1.0		5.56m-z	4.85s-\]	4.73u-\]	4.04z[\]	5.85k-w	<b>5.006g</b>
0.0	0.25	5.83k-w	7.88a-g	5.04q-\]	3.09]	6.75e-p	<b>5.718def</b>
0.25		4.93r-\]	7.82a-h	4.8t-\]	4.08y-]	6.7f-p	<b>5.666defg</b>
0.5		6.27h-u	6.75e-p	5.74k-w	2.93]	6.75e-p	<b>5.688defg</b>
1.0		6.52g-q	6.28h-u	5.2p-\]	3.8[\]	6.36g-t	<b>5.632defg</b>
0.0	0.5	4.93r-\]	6.88e-o	4.36w-]	4.15x-]	6.88e-o	<b>5.440efg</b>
0.25		4.35w-]	9.3a	5.39n-z	4.98q-\]	7.03d-m	<b>6.210bcd</b>
0.5		5.74k-w	7.83a-h	5.32o-\]	6.1i-u	5.74k-w	<b>6.146cde</b>
1.0		7.19d-k	6.5gh-r	5.47m-z	4.79t-\]	6.94d-n	<b>6.178cd</b>
0.0	1.0	5.68k-x	5.26p-\]	6.39g-s	7.18d-l	8.81abc	<b>6.664abc</b>
0.25		6.73e-p	7.6c-i	6.26h-u	5.17p-\]	9.24ab	<b>7.000a</b>
0.5		6.12i-u	6.65f-p	7.17d-l	7d-m	7.52c-j	<b>6.892ab</b>
1.0		6.17i-u	5.66k-y	5.6l-z	7.68b-i	8.49abcd	<b>6.720abc</b>
<b>Cultivar mean</b>		<b>5.844b</b>	<b>7.044a</b>	<b>5.350c</b>	<b>4.927d</b>	<b>6.908a</b>	

“Means followed by the same letter in the same column are not significantly different according to Fisher’s LSD test at  $P \leq 0.05$ . Symbols such as g-t, a-e, etc., indicate statistical groupings based on the test results.”

**Table (6):** Influence of KIN and NAA concentrations on leaf number per jar in micropropagated potato cultivars.

Treatment		Cultivars					Treatment mean
KIN (mg/l)	NAA (mg/l)	Cara	Spunta	Silana	LR	Hermes	
0.0	0.0	26.9k-w	37c-h	30.1f-t	35.4c-k	35.6c-k	<b>33b</b>
0.25		36.3c-i	40bcde	38.7b-f	21.32t-x	53.3a	<b>37.92a</b>
0.5		36c-j	32.5e-q	34.4c-m	35.12c-l	53.3a	<b>38.26a</b>
1.0		46.7ab	21.5s-x	30.4f-s	22.48r-x	41.2bcde	<b>32.46bc</b>
0.0	0.25	30.8f-r	25.5m-x	24.2o-x	21.89r-x	40.7bcde	<b>28.62cd</b>
0.25		17.2x	33.35d-n	22r-x	33.7c-n	40.2bcde	<b>29.29bcd</b>
0.5		32.7e-o	33.1d-o	29h-u	22.5r-x	33.1d-o	<b>30.08bc</b>
1.0		30.6f-r	30.9f-r	32.6e-p	29.3g-u	34.5c-m	<b>31.58bc</b>
0.0	0.5	22.6r-x	42.7bc	28.5h-v	26.1l-x	42.7bc	<b>32.52bc</b>
0.25		22.5r-x	24.7n-x	30.2f-t	27.2j-w	40.6bcde	<b>29.04bcd</b>
0.5		28.4h-v	36.2c-j	30.13f-t	30f-t	28.4h-v	<b>30.63bc</b>
1.0		23.5q-x	29.3g-u	29.6g-t	28h-w	34.8c-l	<b>29.04bcd</b>
0.0	1.0	19.3wx	23.6p-x	23.3r-x	33.2d-o	26.9k-w	<b>25.26d</b>
0.25		19.9vwx	28.1h-w	35.1c-l	41.9bcd	27.2j-w	<b>30.44bc</b>
0.5		20.4uvwx	22.7r-x	36.5c-i	47ab	28.9h-v	<b>31.1bc</b>
1.0		24.2o-x	21.4s-x	38.11b-g	33.3d-n	27.5i-w	<b>28.9cd</b>
<b>Cultivar mean</b>		<b>27.38c</b>	<b>30.16b</b>	<b>30.8b</b>	<b>30.53b</b>	<b>36.81a</b>	

“Means followed by the same letter in the same column are not significantly different according to Fisher’s LSD test at  $P \leq 0.05$ .”



**Table (7):** Number of roots of micropropagated potato cultivars in response to different KIN and NAA concentrations.

Treatment		Cultivars					Treatment mean
NAA (mg/l)	KIN (mg/l)	Cara	Spunta	Silana	LR	Hermes	
0.0	0.0	20j-s	16.67l-s	10.33m-s	21.67h-s	13.33l-s	<b>16.4de</b>
0.5		38c-m	47b-j	26.67e-s	26.67e-s	37.23c-m	<b>35.11bc</b>
1.0		24.67e-s	57.67abcd	45.33b-k	24.33e-s	40.8b-l	<b>38.56b</b>
2.0		34c-p	52bcde	36c-n	0s	25.57e-s	<b>29.51bc</b>
0.0	0.1	32.67d-p	20.67i-s	9n-s	49.33b-h	34.67c-p	<b>29.27bc</b>
0.5		84a	46.33b-j	37.33c-m	51.67b-f	60.67abc	<b>56a</b>
1.0		33c-p	38c-m	35c-p	8.333n-s	26.43e-s	<b>28.15bcd</b>
2.0		12.67m-s	48.33b-i	29e-r	7.667o-s	22.9g-s	<b>24.11cde</b>
0.0	0.5	68.33ab	30d-r	4qrs	44.67b-k	47.67b-j	<b>38.93b</b>
0.5		28.33e-r	46.33b-j	17.67k-s	26.67e-s	30.7d-r	<b>29.94bc</b>
1.0		31.33d-q	50b-g	10.67m-s	24fg-s	35.1c-o	<b>30.22bc</b>
2.0		3.333rs	23.67g-s	7.333pqrs	23.33g-s	14.03l-s	<b>14.34e</b>
<b>Cultivar mean</b>		<b>34.19a</b>	<b>39.72a</b>	<b>22.36c</b>	<b>25.69bc</b>	<b>32.42ab</b>	

“Means followed by the same letter in the same column are not significantly different according to Fisher’s LSD test at P ≤ 0.05.”

**Table (8):** Root length of micropropagated potato cultivars in response to different KIN and NAA concentrations.

Treatment		Cultivars					Treatment mean
NAA (mg/l)	KIN (mg/l)	Cara	Spunta	Silana	LR	Hermes	
<b>0.0</b>	<b>0.0</b>	5.333b-k	5b-l	3.333g-p	6.667a-i	5bc-l	<b>5.067bcd</b>
<b>0.5</b>		7.167a-f	5.833b-j	7.667abcd	6.667a-i	6.567a-i	<b>6.78a</b>
<b>1.0</b>		3.667e-p	7.333a-e	5.667b-j	1.667k-p	5.133b-k	<b>4.693bcde</b>
<b>2.0</b>		1.167mnop	6a-j	5.333b-k	0p	3.233h-p	<b>3.147e</b>
<b>0.0</b>	<b>0.1</b>	7.333a-e	4d-o	4.333c-n	8abc	4.4c-n	<b>5.613abc</b>
<b>0.5</b>		4.667b-m	5.333b-k	5.667b-j	5.667b-j	5.233b-k	<b>5.313abc</b>
<b>1.0</b>		2.5j-p	5b-l	4.333c-n	2.333j-p	3.267g-p	<b>3.487de</b>
<b>2.0</b>		0.3333op	8abc	5.667b-j	2.667j-p	3.667e-p	<b>4.067cde</b>
<b>0.0</b>	<b>0.5</b>	7a-g	6a-j	2.667j-p	7.667abcd	6.9a-h	<b>6.047ab</b>
<b>0.5</b>		4d-o	8.333ab	5b-l	5b-l	5.267b-k	<b>5.52abc</b>
<b>1.0</b>		3i-p	9.667a	3i-p	2.667j-p	5.133b-k	<b>4.693bcde</b>
<b>2.0</b>		0.6667nop	7.667abcd	6.667a-i	1.333l-p	3.5f-p	<b>3.967cde</b>
<b>Cultivar mean</b>		<b>3.903b</b>	<b>6.514a</b>	<b>4.944b</b>	<b>4.194b</b>	<b>4.775b</b>	

“Means followed by the same letter in the same column are not significantly different according to Fisher’s LSD test at P ≤ 0.05.”

**Table (9):** Plantlets acclimatization results of potato cultivars after four weeks.

Cultivar	Surviving	Shoot length	Number of leaves	Number of roots	Root length
Silana	92b	14.2b	11a	11.6a	7.2ab
Hermes	92b	20a	10.4a	13.4a	9.6a
Cara	88c	16b	10.4a	10a	5b
Lady Rosetta	96a	13.8b	9.2a	8.2a	5.4b
Spunta	96a	14.8b	11.6a	9.4a	8.2ab
LSD	<b>0.424</b>	<b>3.62</b>	<b>3.968</b>	<b>5.386</b>	<b>3.581</b>

**Table (10):** Effect of potato genotype on *in vivo* growth and development of plantlets during minitubers production process.

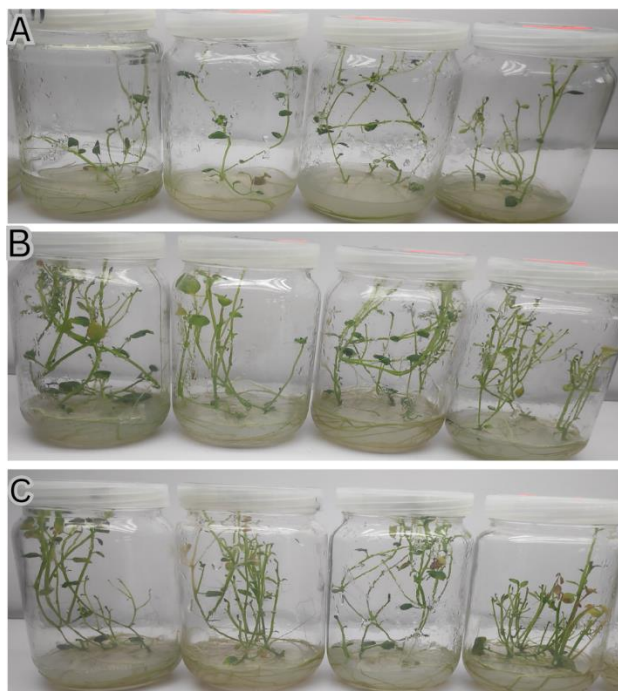
Cultivar	Surviving %	Number of shoots	Shoot length (cm)	Number of nodes	Number of leaves
Cara	33.33bc	2.333b	42.4bc	15c	19c
Spunta	20c	1b	29.33c	8.667c	10.67c
Lady Rosetta	53.33b	2.667b	60.23a	26.67b	32.67b
Hermes	93.33a	5.667a	53.9ab	40.33a	51.33a
LSD	<b>23.07</b>	<b>2.283</b>	<b>17.75</b>	<b>10.85</b>	<b>11.84</b>

**Table (11):** Effect of potato genotype on formation and growth of minitubers produced *in vivo*.

Cultivar	Surviving %	number of tubers/ plant	Weight (g)	Volume (cm <sup>3</sup> )
Cara	60c	10.2a	21.6b	21.8b
Spunta	80b	2.2b	5.12c	5.8c
Lady Rosetta	100a	7.6a	40.2a	38.6a
Hermes	80b	6.8ab	41.56a	36.2a
LSD	<b>0.4358</b>	<b>5.155</b>	<b>12.58</b>	<b>12.86</b>

**Table (12):** Influence of potato genotype on *in vivo* development and growth of super elite tubers.

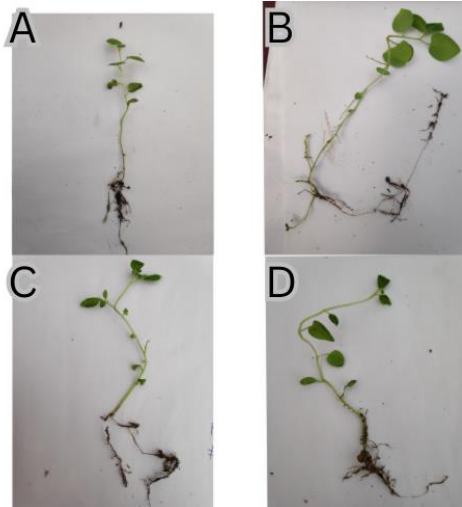
Cultivar	number of tubers/ plant	Weight (g)	Volume (cm <sup>3</sup> )
Cara	9.1a	907.5a	845.5a
Spunta	9.6a	646.4b	605b
Lady Rosetta	8.9a	407c	391c
Hermes	10.7a	480.8c	469c
LSD	<b>2.222</b>	<b>99.24</b>	<b>90.5</b>



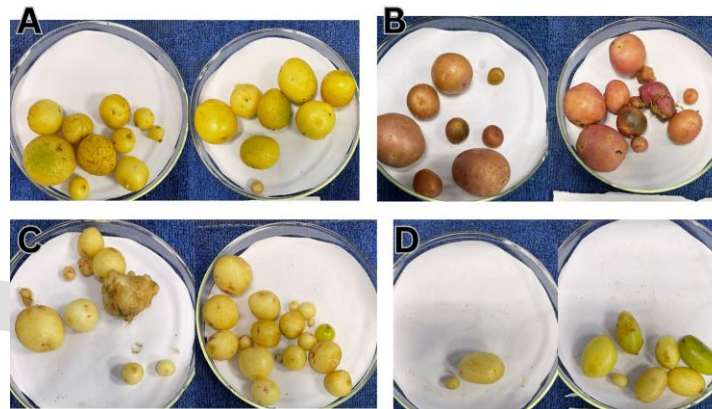
**Figure 1:** Effect of growth regulators on shoot number from *in vitro* growth explants of Hermes cultivar, (A) control treatment ( free hormon media), (B) treatment of 0.25 mg/L KIN and (C) treatment of 0.5 mg/L KIN .



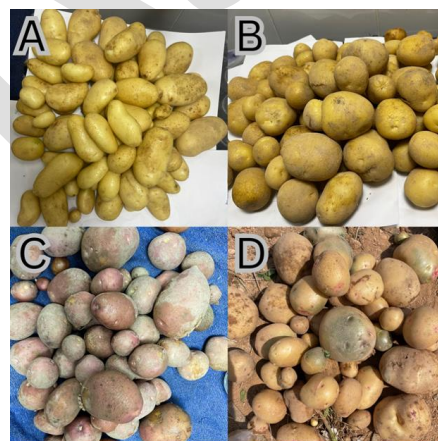
**Figure 2:** Acclimatization Stage of Potato Plantlets: Transition from *In vitro* Culture to Greenhouse Conditions.



**Figure 3:** Plantlets acclimatization of potato cultivar, (A) Silana cultivar, (B) Hermes cultivar, (C) Cara cultivar and (D) Lady Rosetta Cultivar.



**Figure 4:** Comparison of Minituber Production Across Potato Cultivars, (A) Hermes cultivar, (B) Lady Rosetta cultivar, (C) Cara cultivar and (D) Spunta cultivar.



**Figure 5:** Super Elite Potato Production, Comparative Yield of: (A) Spunta cultivar, (B) Hermes cultivar, (C) Lady Rosetta cultivar and (D) Cara Cultivar.