

Article

In vitro Microtuber Formation in *Helianthus tuberosus* and Field Evaluation of Propagation Materials

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Abstract: *Helianthus tuberosus* L. is an important crop for agricultural, medicinal, and industrial uses. Its tubers are rich in inulin, and it is cultivated for food and animal feed. This study was conducted in the Potato and Vegetatively Propagated Vegetables Research Department, Dokii, in collaboration with the Sakha Horticulture Research Station, Kafr El-Shaikh, Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt, during 2022 and 2023. The current study aimed to establish seed production protocol for *H. tuberosus* using tissue culture; Fusaeu cv. tubers were used to establish *in vitro* plantlets cultures. Micropropagation by stem cuttings was conducted on MS media without growth regulators. Microtuberization was investigated using MS media containing 80 g sucrose and four concentrations of paclobutrazol (0, 45, 60, 90 mg/l). The media containing only 80 g sucrose gave the highest tuberization ratio, microtuber number, microtuber weight per container, and average microtuber weight. In an open-field experiment, different propagation material sources (*in vitro* plantlets, minitubers and traditional seed tubers) were evaluated. Traditional seed tubers recorded the highest values of vegetative growth and yield, followed by acclimatized plantlets derived from tissue culture, with no significant differences between them. Minitubers showed the lowest values for growth, tuber yields, tuber dry matter and inulin content.

Keywords: Jerusalem artichoke, *in-vitro* propagation, Microtuberization, Paclobutrazol, traditional tuber seeds, seedling.

1. Introduction

Helianthus tuberosus L (Jerusalem artichoke) is a versatile plant known by various names in different countries, including Jerusalem artichoke, tartufi, sun root, topinambour, and girasole artichoke, it belongs to the Asteraceae family, which includes the sunflower genus. It is a perennial plant native to North America with wide ecological adaptability, but it is grown as an annual crop. *H. tuberosus* tubers, rich in inulin, are harvested for human and animal consumption. Beyond its use as a food source, this plant possesses medicinal properties, with its bioactive compounds showing potential as an innovative

treatment for Alzheimer's disease (Lv *et al.*, 2019; Manokhina *et al.* 2022; Hussien *et al.*, 2023; Zhu *et al.*, 2023). *H. tuberosus* can be propagated through various methods, including *in vivo* techniques using tubers and stem cuttings, as well as *in vitro* methods utilizing tissue culture. Micropropagation, another term for *in vitro* propagation, involves the regeneration of plants in a controlled, sterile environment using a synthetic growth medium.

Micropropagation is one of the most common applications of plant tissue culture. This technique enhances genetic improvement and commercial production of plants. The aseptic cultivation of plant cells and tissues under defined physical and chemical conditions *in vitro* is a critical step in the application of plant tissue culture studies for genetic manipulation and commercial crop production (Samadder & Arumugam, 2023; Bello-Bello *et al.*, 2025). Optimizing the *in vitro* clonal propagation protocol is essential for large-scale reproduction and biotechnological applications in *H. tuberosus* production (Zhang *et al.*, 2023).

Paclobutrazol (PBZ), a plant growth regulator belonging to the triazole family, has demonstrated the ability to ameliorate different environmental stresses by inhibiting the gibberellin biosynthesis, suppressing vegetative growth, and redirecting nutrients to the storage organs (El-Sayed & Shehata, 1996; Tekalign & Hammes, 2004; Moreno *et al.*, 2011; Tesfahun, 2018; Desta & Amare, 2021). Different studies show that PBZ significantly affects potato plants, influencing their height, the number of axillary shoots, number and fresh weight of tubers per plant, and tuber diameter (Dalimunthe *et al.*, 2021). Also, PBZ has been widely used as a growth inhibitor in ornamental plants to enhance tuber development in both laboratory and greenhouse settings (Christiaens *et al.*, 2015; Phasri *et al.*, 2019; Wu *et al.*, 2019). Furthermore, the application of PBZ promotes *in vitro* initiation of potato microtubers (Simko, 1994).

Conventional methods for keeping tubers for propagation have several drawbacks, including prolonged land occupancy for nearly three months, from December to early or mid-March, which negatively affects the cultivation of summer crops. In addition, storing seed tubers under refrigerated conditions increases production costs and may result in the loss of a portion of the seed tubers due to the extended storage period. As an alternative method for the traditional seed tubers, seedlings and microtubers produced by tissue culture techniques of *H. tuberosus* could be a promising propagation method. The objective of the current study is to study the effect of paclobutrazol application on *H. tuberosus* microtuberization and plantlets acclimatization. Additionally, it investigates the impact of using seedlings and minitubers derived from tissue culture compared with traditional seed tubers on vegetative growth, yield, and tuber quality characteristics.

2. Materials and methods

This study was conducted in the Potato and Vegetatively Propagated Vegetables Research Department, Dokii, in collaboration with the Sakha Horticulture Research Station, Kafr El-Shaikh Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt, during 2022 and 2023.

2.1. Plant material and Explant Disinfection

The tubers of *H. tuberosus* Fusaeu cv. were sprouted (Fig. 1a), and these sprouts were collected in September 2022 as a source of explants. Subsequently, the surface of the vegetative sprouts was sterilized by immersion in a 1% sodium hypochlorite solution with the addition of two drops of Tween 20 for 20 minutes. Following sterilization, the sprouts were rinsed three times using sterile distilled water. Under sterile conditions (laminar flow hood), Meristem tips were obtained using a stereomicroscope by excising the meristem tip with two leaf primordia. These meristem tips were inoculated in culture tubes containing MS (Murashige and Skoog, 1962) salts and vitamins medium,

supplemented with 10 mg/l adenine sulfate, 5 mg/l calcium pantothenate, 0.1 mg/l GA₃, 30 g/l sucrose, and 7 g/l agar. The pH was adjusted to 5.7 and autoclaved at 1.45 Kg/cm² for 20 minutes.

The obtained plantlets (**Fig.1b& 1c**) were subcultured using single-node cuttings on MS medium without growth regulators with 4-week subculture intervals. The microtuberization was investigated on four concentrations of paclobutrazol (0, 45, 60, 90 mg/l). The nutrient medium contained MS salts and vitamins, plus 80 g sucrose, 7 g agar, and 10 g charcoal (**Fig.1C**). The pH was adjusted to 5.7 with KOH and HCl before autoclaving at 121 °C for 20 minutes.

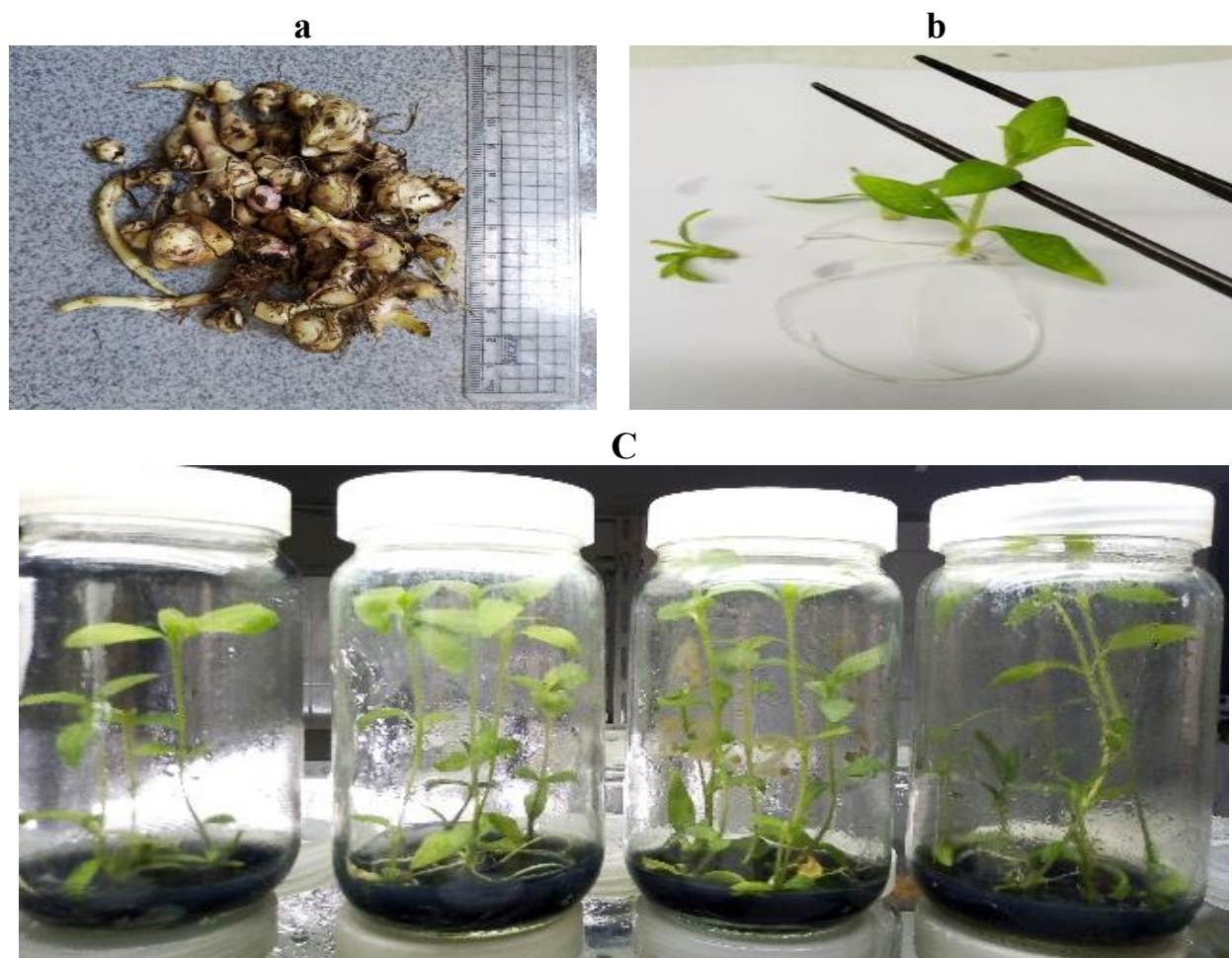


Fig. (1). *In vitro* propagation stages of *H. tuberosus*: a. sprouted tubers; b. produced plantlets; C. growth of nodal cuttings after 4 weeks

A complete randomized arrangement with three replications was used. Each replicate consisted of 10 containers (400 g glass jars containing 35ml/ jar media), and each jar contained 5 explants. The cultures were incubated for two weeks under controlled conditions (24±2°C, 16 light/8 dark, and 80% relative humidity). Following this initial incubation, cultures were transferred to complete darkness for an additional eight weeks. After 10 weeks, the microtuberization ratio, the number of microtubers, and their weight were recorded (**Fig.2A and B**). Obtained acclimatized plantlets and minitubers derived from microtubers were subsequently cultivated in the open field experiment (**Fig.2C and D**).

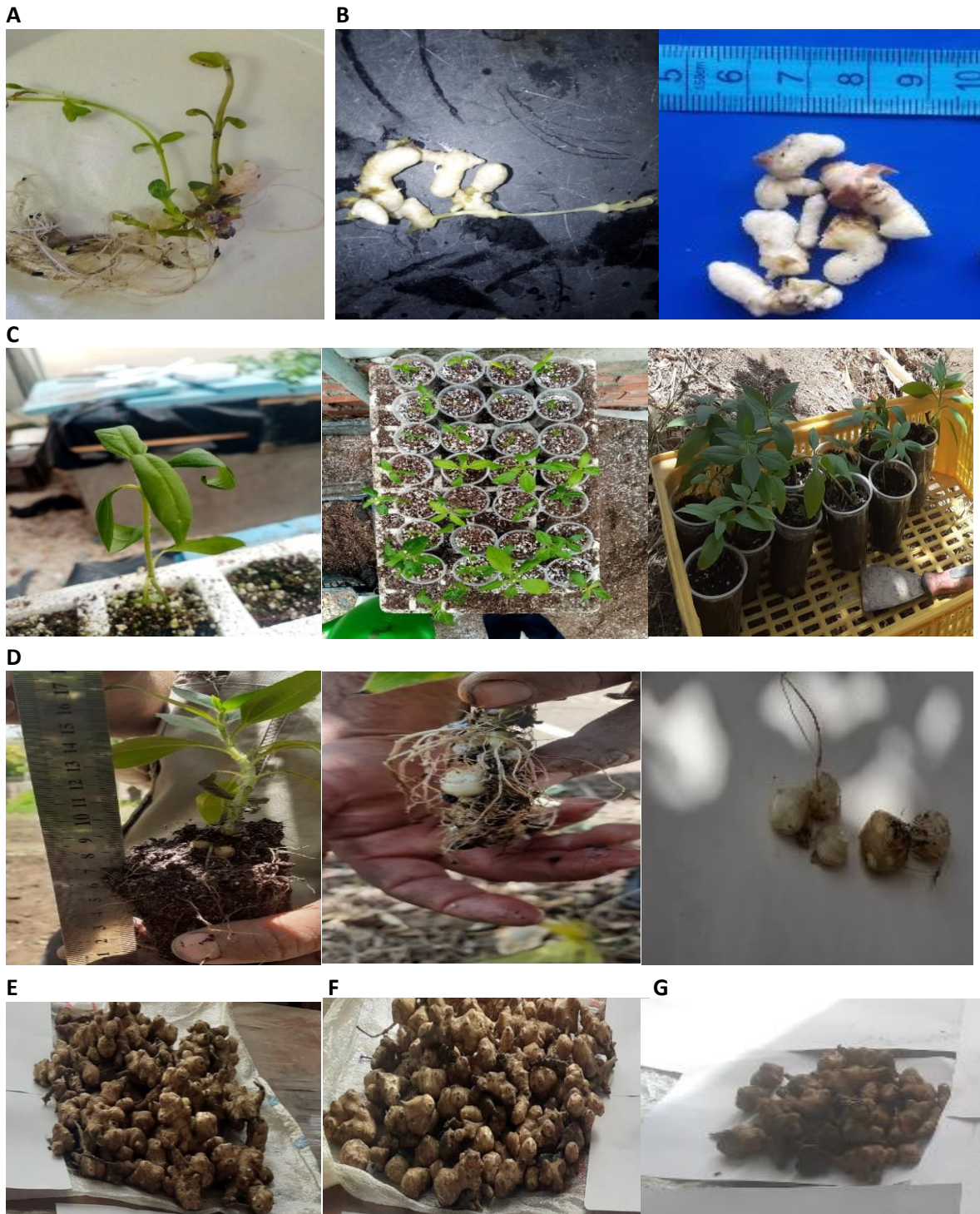


Fig. (2). Plantlet (A), Microtuber (B), *H. tuberosus* acclimatized plantlets (C), Minituber (D), Tubers from Traditional seed tubers (E), Tubers from seedling (F), and Tubers from minitubers (G)

2.2. Field evaluation

An open-field experiment was conducted in April 2023 to evaluate the performance of *H. tuberosus* using different sources of planting material, i.e., traditional seed tubers, *in vitro*-acclimatized plantlets (seedlings), and minitubers (produced from microtubers). Each experimental area consisted of three ridges, each 5 m long and 0.70 m wide, resulting in an approximate plot area of 10.5 m², and *H.*

Tuberoses seeds were planted at 0.40 m spacing. The field-grown plants received the standard agricultural treatments, including cultivation, irrigation, fertilization, and pest management. Collected data of planting included plant length (cm), number of branches plant⁻¹, tuber weight (g), tubers weight plant⁻¹ (kg). Tuber dry matter content (DM, %) was obtained by drying the tuber slices at 70 °C for 72 hours. Inulin content was determined in tubers according to the method of **Winton and Winton (1958)**.

2.3. Statistical Analysis

Data were submitted to analysis of variance (ANOVA) and treatment means were compared using the L.S.D. at 5 % level of probability using SPSS software (IBM SPSS). Charts were created in Microsoft Excel 2016.

3. Results and Discussion

This study adopts an integrated approach combining both *in vitro* and field evaluation. For developing a protocol for microtuber induction in *H. tuberosus* under tissue culture conditions and comparing planting source (plantlets and minitubers versus traditional seed tubers).

3.1. Effect of paclobutrazol concentration on microtuber induction

After 10 weeks, microtubers of *H. tuberosus* were produced on the four examined media, which contained 80 g sucrose with paclobutrazol (PBZ) at different concentrations. The medium containing 80 g sucrose without paclobutrazol produced the highest microtuberization ratio, with a gradual decrease in tuberization ratio with increasing PBZ concentration (**Fig. 3**); the lowest microtuberization ratio was obtained when the highest concentration of paclobutrazol (90 mg/l) was included in the medium.

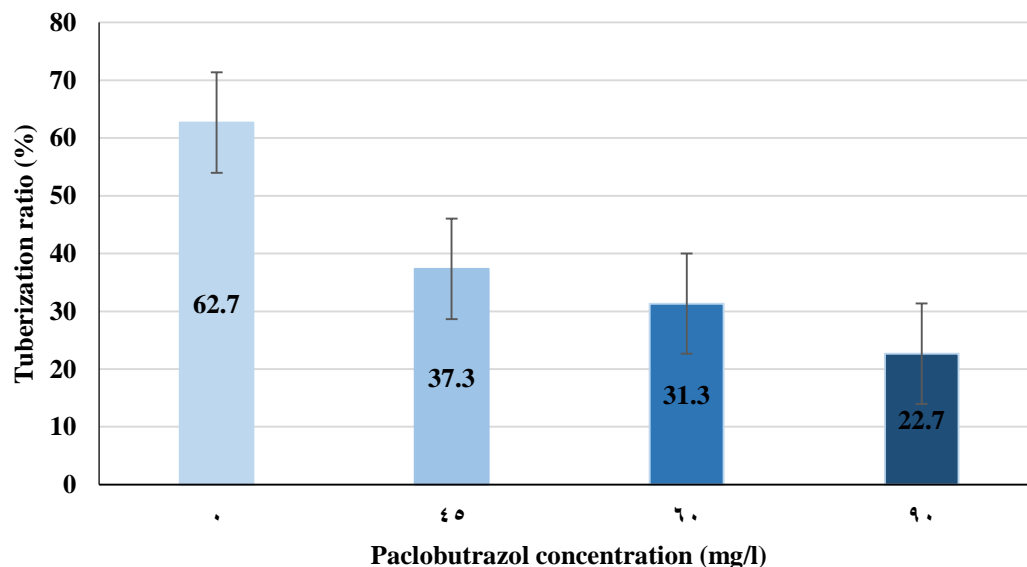


Fig. (3). Tuberization ratio (%) after 10 weeks of culture *in vitro* of *H. tuberosus* on media containing 0, 45, 60, 90 mg/l paclobutrazol. Error bar indicates LSD ($p > 0.05$)

Nutrient medium containing a high concentration of sucrose without paclobutrazol produced the highest microtuber number/jar and microtuber weight/jar and average microtuber weight (Table 1). Increasing PBZ caused a significant reduction in microtuber number and weight.

Table (1). Effects of paclobutrazol concentration on *H. tuberosus* microtuberization after 10 weeks of culture

Paclobutrazol concentration	Microtuber No./ jar	Microtuber weight/jar (g)	Average microtuber weight (g)
Zero	3.3	3.1	1.0
45 mg/l	1.9	1.1	0.6
60 mg/l	1.6	0.7	0.5
90 mg/l	1.1	0.3	0.3
LSD at 0.05	0.3	0.3	0.13

The obtained results indicated superiority for the media that contained 80 g sucrose only without the addition of PBZ. Lower concentration of PBZ produced significantly better microtuberization ratio, microtuber number, and microtuber weight compared with higher concentrations. Sucrose, as a carbohydrate, is one of the most important components of plant tissue culture media it is a carbon source provide energy to plants as plants growing *in vitro* are considered photo mixotrophic, an alternative to *ex vitro* growing autotrophic plants (Kozai, 1991). Moreover, sucrose plays an osmoregulatory role in nutrient media. Also, in potato microtuberization, sucrose provides assimilated carbon converted to starch required for microtuber growth (Khuri & Moorby, 1995). In agreement with the obtained results, *H. tuberosus in vitro* microtubers were obtained on nutrient medium containing 80- 100 g/l sucrose (Gamburg *et al.*, 1999; Homsuwan *et al.*, 2021).

Similar to the obtained results in the current study, PBZ has effectively inhibited the growth of different plant species, including potato (Phasri *et al.*, 2019). PBZ reduced potato shoot growth due to the reduced gibberellin, resulting in a reduction in cell proliferation, causing a reduction in leaf expansion (Tekalign & Hammes, 2004). Furthermore, PBZ resulted in lower plant height (Lim *et al.*, 2004) and reduced plant growth (Rezaei *et al.*, 2017). In the current *in vitro* study, PBZ did not enhance microtuberization and microtuber characteristics when included in the nutrient medium containing 80 g sucrose. A preliminary experiment was conducted using 0, 2.5, 5, 10, 15 and 45 mg/l of PBZ; only 45 mg/l PBZ produced microtubers. In the current study, we used 80 g sucrose without or with 45, 60, and 90 mg/l PBZ. The treatment with 80 g/l alone gave the highest microtuberization rate, microtuber number, and weight. Although PBZ accelerated potato microtuber initiation in lower concentrations, the higher concentrations (10- 1000 mg/l) strongly decreased microtuber number and weight (Simko, 1994). However, Polsa and Ngampanya (2015) studied *in vitro* tuberization of *Helianthus tuberosus* L. using microtuberization medium containing 5 mg/l BAP+ 80 g sucrose+ (500 ppm) of chlorochlorine chloride (anti-gibberellin). The different responses to PBZ in the different studies could be due to the different plant species, cultivar, and concentration used.

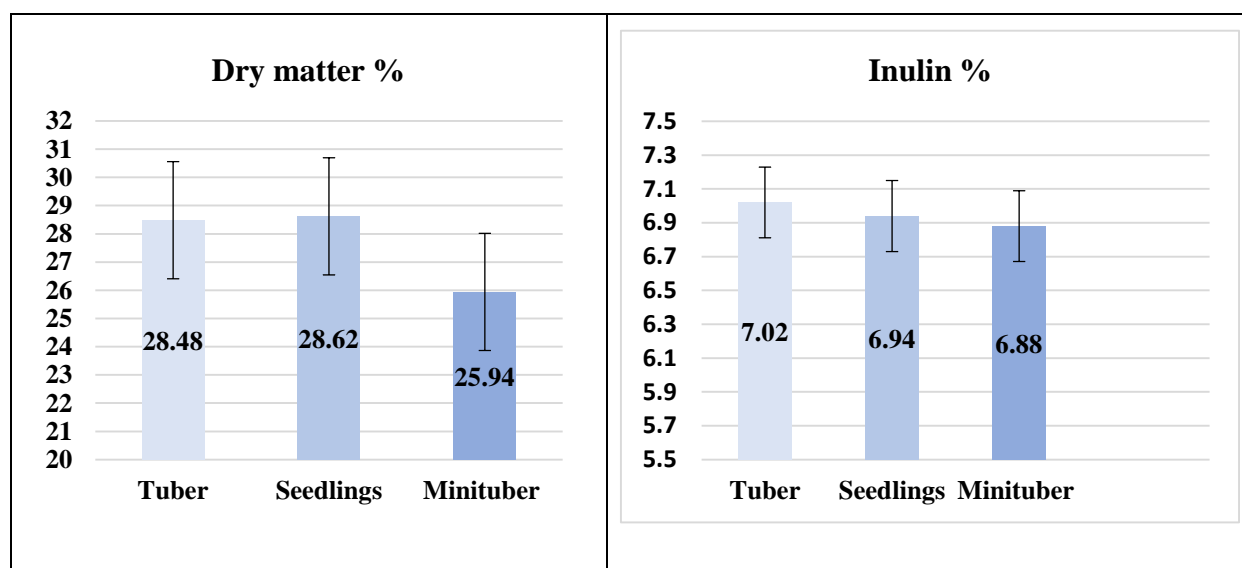
3.2. Plant evaluation in the Field

The evaluation of the three *H. tuberosus* seed sources (acclimated *ex vitro* plantlets, minitubers and traditional seed tubers) was planted in the open field. All three sourced plants grew normally and vigorously with a similar phenotype after two months of planting in the soil. Data in Table 2 and Figure 4 illustrate the effect of the different sources on *H. tuberosus* traits.

Table (2). Effect of traditional tuber seeds, seedlings, and minitubers derived from tissue culture on *H. tuberosus* traits

Treatment	Plant length (cm)	Number of branches plant ⁻¹	Tuber weight (g)	Tuber weight plant ⁻¹ (kg)
Traditional tuber seeds	256.33	7.00	75.69	3.01
seedlings	239.67	7.67	68.00	2.96
Minitubers	139.00	1.33	30.82	0.47
LSD at 0.05	43.17	3.34	15.87	0.451

The obtained data indicate clear and statistically significant effects of propagation method on most growth and yield traits (Table 2). The traditional seed tubers produced the greatest plant length (256.33), tuber weight (75.69 g), and total yield (3.01 kg plant⁻¹), followed closely by the acclimatized *in vitro* plantlets, while the minitubers showed markedly reduced performance. The highly significant differences for plant length, tuber weight, and yield per plant confirm that the propagation method strongly influences overall productivity. Furthermore, the tuber dry matter percentage followed the same trend with superiority for traditional seed tubers and plantlets, while minitubers produced the lowest values for dry matter (Fig 4). Meanwhile, there were significant differences in the inulin percentage. The obtained results agree with findings reported by **Ranalli *et al.*, (1994)**; **Haverkort and Struik (2015)**, who highlighted the superiority of conventional seed systems under field conditions.

**Fig. (4). Effect of traditional tuber seed, seedlings, and minitubers on dry matter and inulin (%) of *H. tuberosus* tuber. Error bar indicates LSD ($p > 0.05$)**

The superior performance of traditional tuber seed and plants can be attributed to the higher reserve nutrients in conventional seed tubers, which enhance early establishment and vegetative vigor. This is consistent with concepts in plant physiology, where early carbohydrate availability plays a key role in supporting canopy development and assimilate production. In contrast, the reduced growth observed in minitubers-derived plants is likely due to limited stored reserves and slower adaptation under field conditions, as commonly reported by **Ranalli *et al.* (1994)**. Total yield per plant followed the same trend as tuber weight. Similar findings were reported by **Struik & Wiersema (1999)**,

confirming that minitubers often result in lower field productivity during early generations. Seedlings and minitubers can produce inulin that is similar to that found in traditional seed tubers; these results agree with **Homsuwan *et al.* (2021)**. Conventional tubers and seedlings are more effective for maximizing field yield, whereas minitubers are better suited for seed production systems despite their lower initial productivity. Minitubers production is an efficient method for obtaining healthy material that can produce the same valuable substances (inulin) as those found in naturally grown plants. At the same time, minitubers are important because they can be produced year-round and are easy to transport and store.

3. Conclusion

In conclusion, *H. tuberosus* can be propagated using *in vivo* (tubers) and *in vitro* by tissue culture techniques. Media containing a high level of sucrose gave good results for microtuberization. Use of paclobutrazol did not improve tuberization, and higher PBZ concentrations caused a reduction in microtuberization. Use of tissue culture materials offers a possibility for propagation of *H. tuberosus* with pathogen-free materials at the desired planting time. Traditional seeds and *in vitro*-derived plantlets gave the best growth and yield, while minitubers gave the lowest response.

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إنتاج الدرينات الدقيقة لنباتات الطرطوفه (*Helianthus tuberosus*) معمليا والتقييم الحقلية لمواد الإكثار

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الملخص العربي

الطرطوفه من المحاصيل المهمة ذات الاستخدامات الزراعية والطبية والصناعية، تتميز درناتها بارتفاع محتواها من الإينولين، كما تزرع لأغراض التغذية البشرية والعلف الحيواني. أجريت هذه الدراسة بقسم بحوث البطاطس والخضر خضرية التكاثر بالدقي، بالتعاون مع محطة بحوث البساتين بسخا، كفر الشيخ، التابعة لمعهد بحوث البساتين، مركز البحوث الزراعية، خلال عامي 2022 و2023. هدفت الدراسة إلى وضع بروتوكول لإنتاج تقاوي الطرطوفه باستخدام تقنية زراعة الأنسجة؛ حيث استخدم الصنف فيوزا. وتم الإكثار الدقيق للنباتات (Micropropagation) بزراعته العقل الساقية على بيئة MS (موراشيغ & سكوج) الخالية من منظمات النمو. وتم دراسة عملية تكوين الدرينات الدقيقة (Microtubers) باستخدام بيئة MS المحتوية على 80 جم/لتر سكروز وأربعة تركيزات من الباكلوبوترازول (0، 45، 60، 90 ملجم/لتر). أظهرت النتائج أن البيئة المحتوية على 80 جم/لتر سكروز فقط حققت أعلى نسبة لتكوين الدرينات الدقيقة وأكبر عدد وأعلى وزن للدرينات الدقيقة، وكذلك أعلى متوسط لوزن الدرينة. وفي تجربة حقلية، تم تقييم ثلاثة مصادر لمواد الإكثار هي: الشتلات الناتجة من زراعة الأنسجة والدرنات الصغيرة (Minitubers)، ودرنات التقاوي التقليدية. وسجلت درنات التقاوي التقليدية أعلى قيم للنمو الخضري والمحصول ونسبة المادة الجافة في الدرناات ومحتوى الإينولين، يليها الشتلات الناتجة من زراعة الأنسجة بدون وجود فروق معنوية بينهما. في حين أظهرت الدرناات الصغيرة أقل القيم.