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Assessment of Plant Growth Regulators and Carbon Sources on the Germination and Growth Process of Dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers) under *In Vitro* Conditions

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Abstract: There are several studies on the medicinal properties of dandelions (*Taraxacum officinale*), but few studies are aimed at understanding the *in vitro* germination process of this plant to improve its propagation. This research was focused on studying *in vitro* seed germination and development of seedlings under different carbon sources, glucose (GLU) or sucrose (SUC) and its concentrations (1.0–5.5%). Additionally, the effect of supplementation with plant growth regulators (PGRs) was studied, measuring the germination capacity, uncertainty and synchrony. Germination was promoted under low carbon source concentrations (≤2.3%), whereas higher concentrations (≥3.2%) had a detrimental effect on this process. GLU allowed the final germination percentages to be slightly better than SUC. Uniformity and synchrony values improved with the presence of PGRs. Results suggested that the best condition to assess *T. officinale* seed germination is in a medium containing GLU 2.3%, 0.5 mg/L 1-Naphthaleneacetic acid (NAA) and 0.5 mg/L 6-Benzylaminopurine acid (BAP). After germination, the best condition for optimal growth of *T. officinale* seedlings was 1.0% SUC supplemented with 0.225 mg/L of NAA and 3.0 mg/L of BAP for initial shoot development. The survival rate was 97% after greenhouse acclimatization. This new method of germination was implemented for the massive propagation of *T. officinale* for further medicinal studies.

Keywords: dandelion; final germination percent; germination synchrony; *in vitro* germination; seedling vigor index; uniformity of germination

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1. Introduction

There is a great amount of scientific research on the medicinal properties of the *Taraxacum* genus due to the presence of various bioactive compounds present in its tissues and organs [1–3]. However, its germination process has been usually studied concerning the natural colonization in meadows and to eradicate it from industrial crops, and scarce information regarding dandelion seed requirements is available. The germination of dandelion seeds (*Taraxacum officinale*) in natural conditions (for instance, in open fields) is low, probably due to pathogenic infections or physical/physiological damage, which makes seedling establishment difficult, finally reducing the opportunity of using this species commercially as a medicinal plant [4]. Few studies have been performed to understand *Taraxacum* germination, improve *in vitro* techniques and achieve a better propagation process. In this sense, *in vitro* culture systems can be manipulated, allowing standardization in plant production, bioactive synthesis and recuperation, and avoiding unfavorable

compounds, such as soil contaminants and heavy metals, which can be absorbed by the leaves from a particular growing site [5].

Different techniques, either *in vitro* or in the field, have been proposed to improve the germination process and break dormancy in plants of several genres. The environmental control, in which the seeds germinate under the influence of specific components in the germination medium, is the most common approach. Carbon sources are generally chosen because of their osmotic effect [6]; however, it also exerts similar functionality to hormones, through interaction with specific compounds as primary messengers [7]. Several phytohormones, such as gibberellins, auxins, cytokinins, abscisic acid (ABA) and ethylene, have been reported as beneficial for plant growth and yield of several species when they are added to the germination medium because they are necessary for controlling many physiological and biochemical pathways involved in germination and seed dormancy [8].

Despite numerous reports indicating that the addition of different compounds to the germination medium, such as nutrients, plant growth regulators (PGRs) and sugars, among others, are useful to achieve better germination, the addition of some external compounds or the manipulation of the medium can also trigger stress responses [8–11], manifested in different physiological and biochemical processes of plants [12,13].

Because of the latter, when the germination process is under evaluation, not only the effect of the treatment on the germination itself, but the effect on the seedling development (growth prior to acclimatation in greenhouse) should be also considered. Therefore, not only the percentage of germination is important, but also the quality and characteristics of the seedling. Shoot and root development need to be considered because they are "short-term" products from germination, and the conditions in which seeds develop (water, light, temperature, nutrients, etc.) affect the morpho-physicochemical characteristics of the plant in your establishment directly [14]. In this sense, seedling vigor is a quantitative term involving the sum of those properties of the seed that determine the productive performance of the plant that must be measured [15].

For the *Taraxacum* genus, as a well-known medicinal crop, developing strong seedlings after germination is extremely important, because the success of a commercial crop establishment can only be achieved when mature plants are fully grown and harvested. There is not much information in the reports involving the *Taraxacum* genus indicating the seedling performance and survival, shoot or root development or even the success of the final *ex vitro* plant establishment. Therefore, the objective of this study is to determine the optimal culture medium of germination and dandelion seedling establishment that allows us to propagate dandelion efficiently to be used in the development of different products as a medicinal plant.

2. Materials and Methods

2.1. Plant Material

Seeds of *T. officinale* (common dandelion) were obtained in the greenhouse of the Institute of Plant Biology and Biotechnology of the Westphalia Wilhelms University Münster (IBBP-WWU, Münster, Germany) in 2013. *In vitro* experiments were performed at IBBP-WWU and data analysis at the School of Biochemical Engineering of the Pontificia Universidad Católica de Valparaíso (Chile) for 2 years (2016–2017).

2.2. In Vitro Seeds Germination

The pappus was previously removed from the achenes and maintained stored in a paper bag at room temperature (18 ± 2 °C) in a dry environment. Seeds were sterilized with 70% (*v/v*) ethanol for 30 s and soaked for 10 min in 10% (*v/v*) NaOCl solution with a few drops of Twin-20. The seeds were rinsed three times with sterile distilled water and sowed orderly in Petri dishes (20 seed/plate with 1 cm between seeds, 4 plates for treatment). Germination medium consisted in Murashige and Skoog (MS) medium [16] with vitamins (4.4 g/L), agar (7 g/L), carbon sources, glucose (GLU) or sucrose (SUC) and PGRs (1-Naphthaleneacetic acid (NAA) and 6-Benzylaminopurine acid (BAP) in combination) in

18/6 h light/dark at a photosynthetic photon flux density (PPFD) of 200 µmol m^{-2} s^{-1}, according to the medium composition indicated in the experimental design presented in Table 1. PGR and carbon sources were tested in a full factorial designed experiment. Plates were kept in a growth chamber at 18 °C (±2 °C); germination was evaluated daily, and the seed was considered germinated when the embryo broke the seed coat and grew up to 1 mm. The treatments were followed until no germination was observed in a 2-week period. Control treatments was set up using GLU or SUC as a carbon source but without the addition of PGRs. Germination indexes calculated in this work were arranged as germination capacity (final germination percentage (FGP) in %, mean germination time (MGT) in d), germination homogeneity (coefficient of variation of the germination (CVt) in %), germination uncertainty (uncertainty (U) in bit) and germination synchrony (synchrony (Z), unitless) (Table S1; Supplementary Material) [17]. All the chemicals and reagents were purchased from Phytotechnology Laboratories®, Duchefa Biochemie® and Merck®.

Table 1. Plant growth regulators (PGRs; 1-Naphthaleneacetic acid (NAA) and 6-Benzylaminopurine acid (BAP) in combination) and carbon source (CS) type and concentration in the experimental design for Taraxacum's germination studies as proposed in this work.

PGRs [a] (NAA mg/L–BAP mg/L)	CS Type	CS Concentration (%)
0.05–0.5	GLU	1.0
0.05–1.25		2.3
0.05–3.0		3.2
0.225–0.5		5.5
0.225–1.25	SUC	1.0
0.225–3.0		2.3
0.5–0.5		3.2
0.5–1.25		5.5
0.5–3.0		
0.0–0.0		

[a] Each combination of NAA and BAP was tested with a different type of carbon sources, glucose (GLU) or sucrose (SUC) and at different concentrations independently. 10 PGR treatments × 8 carbohydrate treatments, a total of 80 treatments.

2.3. In Vitro Development Seedling

Seedling development was measured through the development of the bud (number and length of leaves) after the third week of germination. Seedling vigor index (SVI, in %·cm) was calculated as the multiplication of FGP and seedling (shoot + root) length [18] at each condition tested. *In vitro* seedling development was followed for a 3-week period after measuring the growth parameters (in total 6 weeks after germination). These new plantlets were maintained in the selected medium for the development of shoots and roots, according to the results obtained in the previous stage. Then, the seedlings belonging to the best treatment obtained in the previous stage were transferred to plastic-coated pots for acclimatization for a period of 3 weeks and, finally, the survival rate was counted.

2.4. Data Analysis

The results collected from field experiments were subjected to statistical analysis using SPSS® software v. 16.0 (IBM, Chicago, IL, USA). The same procedure was used in a previous study allowed us to determine the significant differences between the treatments, with dependent (FGP, CVt, U, Z, SVI, leaves length and leaves number) and independent variables (carbon type and concentration, and PGRs), as indicated in Tables S2 and S3 (Supplementary Material) for germination and seedling growth experiments, respectively. The difference among treatment means ($p \leq 0.05$) was tested for significance using Duncan's multiple range test (DMRT) for both experiments [19].

3. Results

3.1. Germination Capacity

In Tables 2 and 3, FGP for *T. officinale* seeds for the carbon source and PGRs treatments is presented. The carbon source affected the FGP related to its concentration in the medium, showing a decrease in the germination percentage at higher sugar concentration. FGP values were in the range of 50–91%, 57–87%, 37–55% and 24–49%, at 1.0%, 2.3%, 3.2% and 5.5% for GLU and of 46–74%, 50–80%, 39–70% and 25–34% for SUC at the same concentrations, respectively.

Table 2. Final germination percent (FGP) of *Taraxacum officinale* seeds at different concentration of glucose (GLU) and PGRs treatments. Medium supplemented with NAA and BAP (in combinations) at 18 °C and photoperiod 18/6 h light/dark.

PGR Treatments	FGP (%)			
(NAA mg/L–BAP mg/L)	GLU 1.0%	GLU 2.3%	GLU 3.2%	GLU 5.5%
0.05–0.5	70 ± 5.7^{b}	$79 \pm 8.3^{a,b,c}$	51 ± 4.8^{a}	$31 \pm 5.7^{c,d}$
0.05–1.25	$58 \pm 5.7^{c,d}$	$79 \pm 5.6^{a,b,c}$	52 ± 6.9^{a}	$45 \pm 6.9^{a,b}$
0.05–3.0	91 ± 6.1^{a}	86 ± 8.4^{a}	50 ± 5.3^{a}	$40 \pm 5.7^{a,b,c}$
0.225–0.5	50 ± 4.0^{d}	$61 \pm 8.2^{d,e}$	39 ± 8.7^{b}	$48 \pm 4.4^{a,b}$
0.225–1.25	$67 \pm 6.4^{b,c}$	$81 \pm 6.6^{a,b}$	$46 \pm 4.1^{a,b}$	49 ± 8.0^{a}
0.225–3.0	$66 \pm 7.0^{b,c}$	57 ± 3.8^{e}	53 ± 10^{a}	$46 \pm 4.9^{a,b}$
0.5–0.5	88 ± 4.7^{a}	$87 \pm 6.0^{a,b,c,d}$	55 ± 5.3^{a}	24 ± 6.8^{d}
0.5–1.25	72 ± 7.9^{b}	$71 \pm 7.8^{b,c}$	37 ± 4.8^{b}	28 ± 8.2^{d}
0.5–3.0	71 ± 8.6^{b}	$67 \pm 11^{c,d,e}$	54 ± 6.4^{a}	$47 \pm 5.0^{a,b}$
0–0 (control)	68 ± 4.2^{b}	58 ± 8.8^{e}	47 ± 6.7^{a}	$40 \pm 8.7^{b,c}$

Values are means ± SE of 4 replicates, containing 20 seeds each. Letters indicate significant differences from control at $p \leq 0.05$.

Table 3. FGP of *Taraxacum officinale* seeds at different concentration of sucrose (SUC) and PGRs treatments. Medium supplemented with NAA and BAP (in combinations) at 18 °C and photoperiod 18/6 h light/dark.

PGR Treatments	FGP (%)			
(NAA mg/L–BAP mg/L)	SUC 1.0%	SUC 2.3%	SUC 3.2%	SUC 5.5%
0.05–0.5	71 ± 7.0^{a}	$71 \pm 7.0^{a,b}$	$68 \pm 7.5^{a,b}$	30 ± 5.9^{a}
0.05–1.25	69 ± 3.4^{a}	$69 \pm 5.0^{a,b,c}$	70 ± 10^{a}	32 ± 7.6^{a}
0.05–3.0	$53 \pm 5.0^{b,c}$	$77 \pm 4.8^{a,b}$	$68 \pm 5.3^{a,b}$	33 ± 4.4^{a}
0.225–0.5	50 ± 5.6^{c}	$54 \pm 6.4^{d,e}$	$46 \pm 5.2^{d,e}$	27 ± 7.5^{a}
0.225–1.25	51 ± 13^{c}	$65 \pm 6.4^{a,b,c,d}$	$44 \pm 5.2^{d,e}$	29 ± 5.9^{a}
0.225–3.0	48 ± 7.8^{c}	$64 \pm 14^{b,c,d,e}$	$62 \pm 6.4^{a,b,c}$	30 ± 6.6^{a}
0.5–0.5	74 ± 9.8^{a}	80 ± 6.0^{a}	$65 \pm 14^{a,b}$	32 ± 10^{a}
0.5–1.25	$65 \pm 9.6^{a,b}$	$55 \pm 11^{c,d,e}$	39 ± 7.9^{e}	25 ± 4.5^{a}
0.5–3.0	46 ± 8.1^{c}	$75 \pm 7.4^{b,c}$	$48 \pm 8.0^{c,d,e}$	34 ± 3.3^{a}
0–0 ^{b} (control)	$53 \pm 7.4^{b.c}$	50 ± 15^{e}	$55 \pm 10^{b,c,d}$	29 ± 19^{a}

Values are means ± SE of 4 replicates, containing 20 seeds each. Letters indicate significant differences from control at $p \leq 0.05$.

There was no clear tendency in the FGP between the different hormonal combinations when one of them remains fixed. However, it was observed that some of them improve germination when they are present compared with the control. In general, higher germination percentage was obtained using a combination of 0.5 mg/L NAA and 0.5 mg/L BAP as PGR treatment, in a medium supplemented with GLU or SUC, indistinctly and despite the sugar concentrations. In terms of the germination velocity, MGT for SUC 2.3% showed values ranging from 8.5 to 21.9 d, for the PGR combinations 0.5 mg/L NAA-0.5 mg/L BAP and 0.225 mg/L NAA–1.25 mg/L BAP, respectively. For GLU 2.3%, MGT values ranged from 13.6 to 21.5 for the same PGR combination as well (data not shown). In general, MGT

values were lower for SUC than GLU, which can mean that under the same hormonal combinations, the contribution of SUC to the culture medium accelerates the germination of dandelion seeds.

The general results of the MANOVA are presented in Table S2 (Supplementary material). The effect of carbon source and PGR treatments studied on *T. officinale* germination was statistically significant regarding the germination process ($p < 0.001$). As shown, a significant interaction between PGR treatments, carbon source type (CS) and carbon source concentration (%CS) on the overall germination process ($p \leq 0.006$) was observed. Only the interaction between PGR and CS were not significant ($p = 0.195$). Considering the effect of the variables (FGP, U, CVt) on the calculated parameters, for all parameters evaluated there were a significant effect, except for the Z index which is not significantly affected by CS ($p = 0.3231$).

3.2. Germination Uniformity and Synchrony

Germination homogeneity, uniformity and synchrony indexes (CVt, U, and Z, respectively) for *T. officinale* seeds for GLU and SUC at 2.3% are presented in Figure 1a–c. As observed, average CVt values were in the range of 38–69% and 43–62% for GLU and SUC, respectively. No statistical difference was observed regarding carbon source and PGR treatments; however, slightly lower results (up to 15% lower) were obtained using SUC as the carbon source compared with the obtained with GLU. On the other hand, CVt values were lower than the control value by up to 20%. U values were in the range of 1.5–1.9 and 1.2–2.1 bit for GLU and SUC at 2.3%, respectively. In general, PGR treatments enhanced this index, by lowering the values compared with the control, up to 30 and 60% lower for SUC and GLU as carbon sources, respectively. Intermediate concentration of PGRs seemed to be the best conditions for having a low U index. Regarding synchrony, Z values were in the range of 0.2–0.3 and 0.2–0.5 GLU and SUC at 2.3%, respectively. Compared with the control, PGR treatments did not enhance the performance of this index. Moreover, PGR treatments lowered this index up to 10 and 20% in GLU and SUC, respectively, specifically at 0.05 mg/L NAA–1.25 mg/L BAP. In general, no clear trend was observed between PGR treatments and these indexes.

Considering CVt, U and Z for SUC 2.3%, it can be affirmed that the treatments when the lowest germination variation occurs were according to the treatments when the lowest average germination time values were obtained, which is completely different for the 2.3% GLU condition. Therefore, for dandelion seeds, it would be more efficient to use 2.3% SUC if we look at these germination parameters.

3.3. Seedling Development

Seedling characteristics varied with the sugar concentration in the medium. The height and color of the seedling were distinct at different sugar concentrations, being smaller and darker at the highest CS content (5.5%), regardless of the presence of GLU or SUC in the medium. In Figure 2a,b, a comparison between seedlings in the 3rd week in 2.3% and 5.5% of SUC is presented. As observed, the leaves of seedlings showed a darker green color and had smaller and thicker leaves (Figure 2a). at 3.2% and 5.5% of the carbon source than the leaves from seedlings grown at 1.0% and 2.3% which showed a lighter color, were softer and had a greater surface (Figure 2b) At the lower CS concentrations (1.0% and 2.3%), true leaves were observed at the 2nd–3rd week of germination, whereas at higher CS concentrations (3.2% and 5.5%), only after the 3rd–4th week, small leaves appeared, but no further seedling growth and normal shoot development were observed.

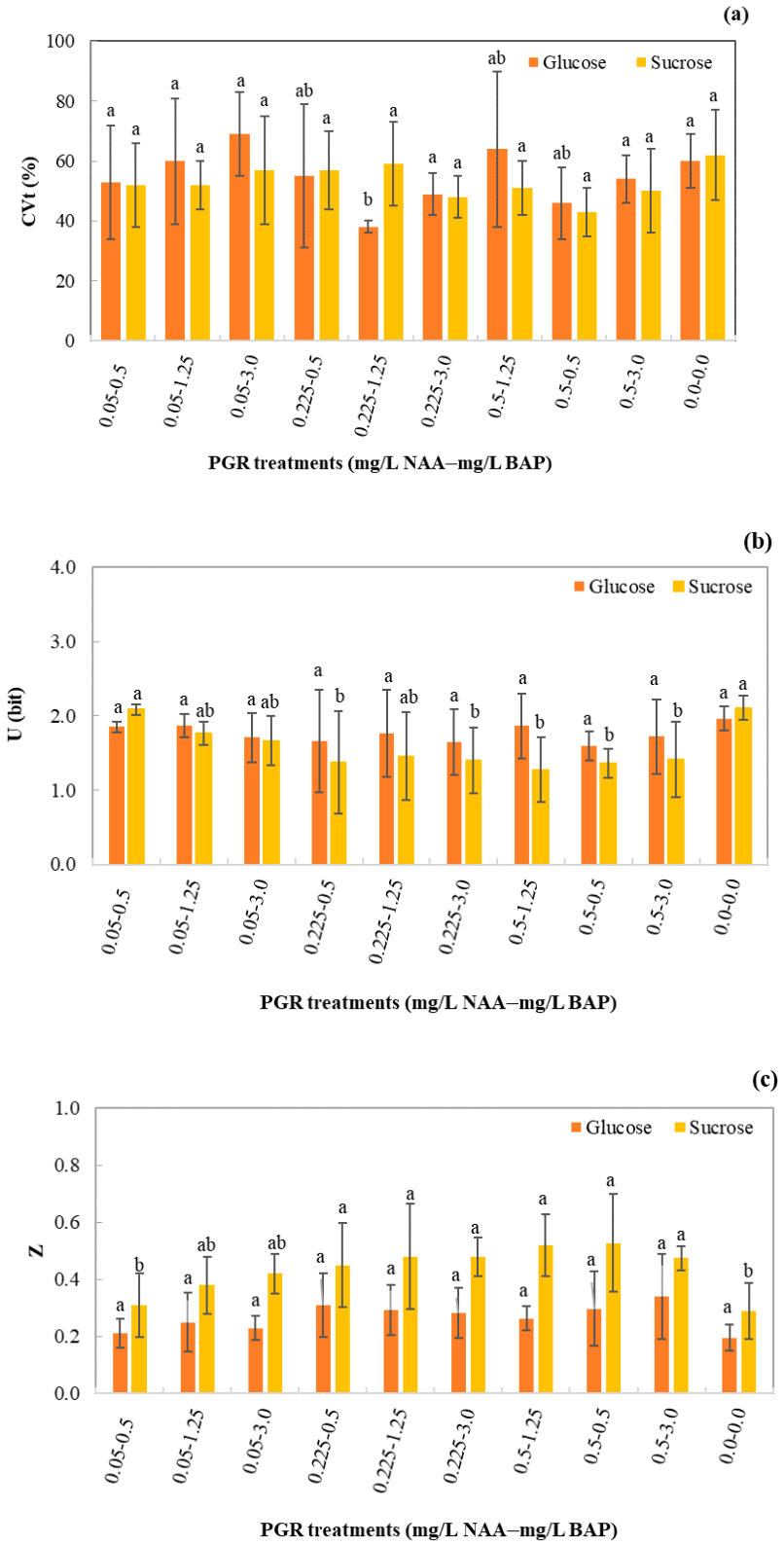

Figure 1. Germination uniformity and synchrony indexes: (**a**) Coefficient of variation of the germination (CVt) values; (**b**) Uncertainty (U) values; (**c**) Synchrony (Z) values of *Taraxacum officinale* seeds at different carbon sources at 2.3% and PGR treatments. Medium supplemented with NAA and BAP (in combination) at 18 °C and photoperiod 18/6 h light. Glucose (GLU); Sucrose (SUC); Plant growth regulators (PGR). Values are means ± SE of 4 replicates, containing 20 seeds each. Letters indicate significant differences from control at $p \leq 0.05$.

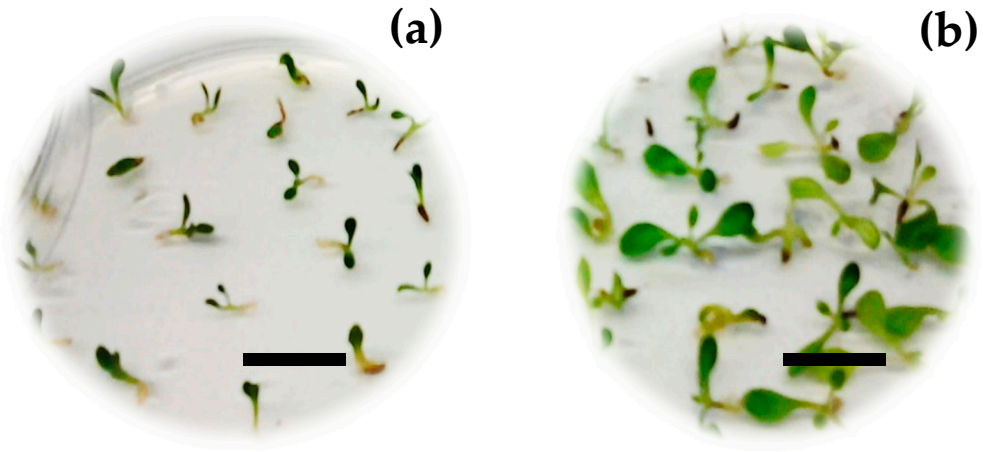

Figure 2. Germination of *Taraxacum officinale* in SUC at (**a**) 5.5% and (**b**) 2.3%. (0.5 mg/L NAA, 0.5 mg L/L BAP, 18/6 h light/dark, 18 ±2.0 °C, two weeks after germination). The size of the bars is 1 cm.

In Figures 3 and 4, the germination of *T. officinale* seeds is presented. As shown, primary root developed within the first 5 days (Figure 3b), the petiole not emerging until the 10th day (Figure 3e), when cotyledons finally appeared, and the seed coat was completely off of the seedling. After root protrusion, cotyledons developed faster when PGRs were present in the germination medium. However, the root increased its length only after cotyledons were fully grown and true leaves were present. At the time of shoot/root measurements (3 weeks after germination), the root was poorly developed for the seeds sowed in the nine PGR treatments proposed (Figures 3g and 4a), showing slow growth for the next weeks. On the other hand, roots increased their length considerably in the control seeds (between 4–6 times the length of the respective shoots, and up to 10 cm) during this period. As observed, *in vitro* growth under controlled conditions allowed to develop suitable organs for further acclimatization in the greenhouse (Figure 4b,c).

Figure 3. Seedling development of *Taraxacum officinale* seeds in 2.3% SUC and 0.225 mg/L NAA-3.0 mg/L BAP at (**a**) 0, (**b**) 5, (**c**) 6, (**d**) 7, (**e**) 10, (**f**) 14 and (**g**) 21 days after sowing (18/6 h light/dark, 18 ±2 °C). The size of the bars is 5 mm.

Regarding seedling development under the different CS and PGR conditions proposed, Table 4 presents the best carbon source condition (2.3% SUC) on the effect on leaf number and length and vigor index of *T. officinale* seedlings under the proposed conditions post-germination. Shoot development (number and length of leaves) was influenced by both carbon sources and RGF treatments ($p < 0.0001$) (Table S3). All values for the different variables under the different treatments can be seen in the tables attached in the supplementary material (Tables S4–S6).

Figure 4. Development of *Taraxacum officinale* plantlets at (**a**) 2 weeks, (**b**) 3 to 8 weeks *in vitro* and (**c**) transferred plantlets 16 weeks after sowing for greenhouse acclimation. The size of the bars is 1 cm.

Table 4. Number of leaves per seedling, leaf length and vigor index of *Taraxacum officinale* seedlings under optimal carbon source (2.3% sucrose), at different treatments of PGRs. Medium supplemented NAA and BAP (in combinations) at 18 °C and photoperiod 18/6 h light/dark in a 3-week period. Sucrose (SUC); Plant growth regulators (PGR); Carbon Source (CS); Seedling Vigour Index (SVI). Letters superscript indicate significant differences from control at $p \leq 0.05$.

PGR Treatments	**CS 2.3% SUC**		
(NAA mg/L–BAP mg/L)	**Number of Leaves per Seedling**	**Leaf Lengh (cm)**	**SVI (%× cm) × 100**
0.05–0.5	5.7 ±0.4 ^{a,b}	3.5 ±0.2 ^{a,b}	2.5 ±0.2 ^{b}
0.05–1.25	4.7 ±0.3 ^{b,c}	3.1 ±0.4 ^{a,b,c}	2.4 ±0.3 ^{b,c}
0.05–3.0	4.7 ±0.8 ^{b,c}	2.6 ±0.2 $^{b.e}$	2.0 ±0.3 ^{b,c,d}
0.225–0.5	5.7 ±1.6 ^{a,b}	3.8 ±0.8 ^{a}	2.0 ±0.3 ^{b,c,d}
0.225–1.25	6.6 ±0.1 ^{a}	2.2 ±0.7 ^{d}	2.6 ±0.6 ^{b}
0.225–3.0	5.0 ±0.4 ^{b,c}	2.7 ±0.6 ^{b,c}	1.7 ±0.3 ^{c,d}
0.5–0.5	4.6 ±1.7 ^{b,c}	3.5 ±0.5 ^{a,b}	1.4 ±0.7 ^{d}
0.5–1.25	4.7 ±0.6 ^{b,c}	2.9 ±0.1 ^{b,c}	1.6 ±0.4 ^{d}
0.5–3.0	4.2 ±0.9 ^{c,d}	2.4 ±0.8 ^{e}	1.8 ±0.2 ^{c,d}
0–0	4.1 ±0.8 ^{c}	2.8 ±0.8 ^{b,c}	5.8 ±1.3 ^{a}

In general, leaf numbers ranged between 2.9 and 7.6 leaves/seedlings considering all the conditions tested from the second week of germination. Leaf number increased with the increment of the carbon source, regardless of the presence of GLU or SUC in the medium, with the exception of concentration 5.5%. For example, leaf number was 16% lower at 5.5% of the carbon source in comparison with the addition at 1.0% of GLU. The highest values were obtained at 1.0% and 2.3% of SUC, incrementing between 60% to 80% seedling development, compared with the control which shoot and root development was considerably high. In these conditions, leaf lengths ranged from 3.3 cm to 6.8 cm and 4.1 cm to 6.6 cm, respectively. Leaf length was up to 60% higher when SUC was added to the medium in comparison with GLU and showing the largest leaves at 2.3% SUC with values ranging from 2.4 cm to 3.8 cm depending on the PGR treatment. When NAA was added at intermediate concentration (0.225 mg/L) and BAP was added at the lower concentrations (0.5 mg/L), the best seedling growth was observed at 2.3% SUC, with up to 35% more leaves. Interestingly, at 5.5% SUC, seedling growth was better was better at the same concentrations mentioned (0.225 mg/L NAA–0.5 mg/L BAP). Compared with the control, most of the PGRs treatment yielded better results, with shoots sometimes duplicating in number as compared with the seedlings grown without PGRs addition. However, the higher values of leaf lengths were obtained when no PGRs were added to the medium.

The Seedling Vigor Index (SVI) was strongly influenced by both CS and PGRs treatments ($p < 0.001$) (Table S3). As shown in Table 4, SVI values were higher for SUC and GLU at 2.3%, when PGR treatment was 0.225 mg/L NAA–1.25 mg/L BAP, with values of 262%*cm and 96%*cm, respectively. In general, this PGR treatment showed lower values than those obtained for the control, especially in the root development. These results may be due to the root growth performance of the CS and PGR treatments, which was much lower than the control treatment. High seed vigor is known to promote growth and increase yields in agricultural production. In this case, SUC promotes twice the emergence of dandelion seeds and allows for uniformity in seedling development better than GLU at the same concentration.

Due to the effect of CS supplementation and PGRs in the medium on seedling development, a three-step seedling production protocol was established. Initially, seeds should be germinated on MS medium containing 2.3% GLU supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP, then transferred to MS medium containing 2.3% SUC with the addition of 0.225 mg/L NAA–1.25 mg/L BAP to promote aerial development and finally transferred to MS medium containing 2.3% SUC lacking PGR for rooting. Under these conditions, the shoots developed sufficiently for proper establishment in the greenhouse. Figure 4c shows the growth of *T. officinale* seedlings maintained at 2.3% SUC before the seedlings were transferred to the greenhouse. Considering the seeds initially sown under the selected conditions, the final survival rate was 97 ± 1%.

Plantlets were monitored for a 2-year period, in which fully functional and healthy plants grew, all generating flowers and seeds, and demonstrating the *in vitro* regeneration of *T. officinale* according to the proposed conditions (data not evaluated).

4. Discussion

Few studies have been carried out regarding the effect of carbon source and other metabolites on the germination process of the *Taraxacum genus*. The first study in this area was the addition of dextrose and salts to the germination medium under different light and temperature conditions. In that experiment, all the conditions improved seed germination compared with the germination in pure water [20], indicating a positive effect of the osmolality of the medium on the germination process.

Recently, some authors studied the effect of SUC on *Taraxacum platycarpum* germination, in which FGP was 50–65% and 30–40% at 1.0% and 3.0% of the carbon source, respectively, and depending on whether the seeds were sowed vertically or horizontally [4]. Because MS concentration was different for both conditions proposed, no direct correlation with the results at 1.0% and 3.0% could be further established. Nevertheless, that study agrees with the results obtained in this work, in which higher carbon source concentrations decrease FGP significantly. The same occurs in the case of PGRs application on T. kok-saghyz lower hormone concentrations of 2,4-D in combination with 1.0 mg/L BA were more efficient for shoot regeneration from seeds. However, since the operating conditions are very different from those used in this work, they cannot be correlated [21].

4.1. Germination Capacity, Uniformity, and Synchrony

Regarding *T. officinale* seed germination, FPG values calculated in this work are in general below those reported for this species, even at control conditions (without PGR added to the medium). FGP values have been reported in the range of 70–100% when the temperature was in the range of 16–20 °C (similar to the proposed in our study), but strongly dependent on it [22–24], also on the light availability [25], sowing [26,27] and season conditions [28].

This was the result of having performed the experiment in an agar matrix instead of the traditional wet disc paper on a Petri dish or in soil pots; therefore, gas and mass transfer interactions need to be considered. Only one work reported the use of an agar matrix for *T. platycarpum* seed germination [4] with FGP values in agreement with those obtained in this study. However, another study in soil indicates 60% of germination for

this species, so the difference between agar matrix and soil, might be important but not conclusive. Seeds age is another variable that influences the germination process [17], but this parameter is scarcely mentioned in the reports and it was not considered in this work. Therefore, direct comparison of FGP values cannot be pursued between studies unless conditions were similar; and results might provide accurate comparisons within a single experiment. Results indicate that the best homogeneity (lower values of CVt) and higher synchrony (lower values of U and higher of Z [17]) can be obtained by supplementing the germination medium with concentrations between 0.225 to 0.5 mg/L of NAA and BAP. CVt, U and Z values have not been studied yet for the *Taraxacum* genus; therefore, this is the first report addressing this subject.

4.2. Effect of Carbon Source and PGRs on Germination

Carbon sources had a significant effect on the germination of *T. officinale* seeds, especially considering the concentration of GLU or SUC in the medium. Low carbon source concentrations showed best germination performance, both in quantity and in quality, with best results when the sugars were present at 1.0 and 2.3% in the germination medium. At higher concentrations (3.2 and 5.5%), fewer seeds germinated, and the seedlings were considerably damaged. Taken into account the nature of the abnormal germination, it can be proposed that sugars were exerting osmotic pressure into the cell. The environmental factors that establish the dynamics of the water relations of the seed determine water retention and its movement [29], and finally the germination process. The osmotic effect varies directly with the presence of compounds present in the culture medium, mainly salts and sugars, the concentration of which determines the availability of water. It has been indicated that high sugar concentrations delay germination and generate abnormal seedlings [4], which is consistent with the observations of this work when high carbon SUC concentrations were studied (3.2 and 5.5%). However, the effect of sugars on germination is not only related to the osmotic pressure, since in tests with other purely osmotic compounds the response on germination is different [30,31], but both sugars modulate critical plant processes such as germination when they interact with phytohormones and PGRs [32,33].

It has been stated that SUC acts as a signaling molecule that regulates genes involved in photosynthesis, metabolism, and developmental processes, whereas GLU, as an anormal product of SUC decomposition, delays germination and affects negative seedling development [6,30,31,34]. Different carbohydrates can generate different effects in morphogenesis; therefore, it is necessary to evaluate independently how it is affected according to the genotype of the species and the specific stage of development. Additionally, there are several factors that determine the effectiveness of a carbon source, such as its type, concentration and mutual interaction [35]. Although SUC is rapidly converted to GLU and fructose, it has been reported that the addition of both sugars in equimolar concentration does not have the same effect on germination, suggesting the preponderant role of SUC as part of signaling pathways [34]. Despite the latter, SUC is widely used in *in vitro* cultures due to its overall performance, availability and cost.

The effects of PGRs have been scarcely studied on the *Taraxacum* genus. For *T. officinale*, seed germination rates were increased at kinetin (Kin) concentration between 0.5–1.5 mM (0.11–0.32 mg/L) compared with the control, with FGP values of 76–84% and 51%, respectively [36]. These values are consistent with those obtained in this work, in which FGP values for the control were up to 68%. Nevertheless, under certain PGRs treatments (between 0.05–3.0 mg/L of NAA and BAP in combination), these values increased up to near 90%. Adding NAA may enhance germination under certain concentrations, as observed in this work, because NAA turns the seed insensitive to ABA, which has an inhibitory effect on germination by delaying radicle expansion; however, NAA interact with ABA pathway and its negative effects are reduced. For instance, individual addition of auxins and cytokinins stimulated the germination and development of *Digitalis purpurea* [18], *Comparettia falcata* [37] and *Paphiopedilum ciliolare* [38]. However, the presence of auxins may

have a negative effect because, in the early stages of germination, auxins are not strictly necessary and may cause an intoxication or competition effect by the receptors of other growth regulators [39]. Cytokines also act during germination and leaf senescence, mitigating cell stress produced by salinity, drought, heavy metals and oxidative stress [8]. This last characteristic may account for the fact that high levels of BAP in the medium (3.0 mg/L) maintain similar germination percentages despite the carbon source concentration (from 1.0% to 5.5%), which was not observed in lower concentrations of BAP (Figure 2a), acting maybe as a stress alleviator. Nevertheless, the effect of NAA and BAP on *T. officinale* seed germination needs to be further studied considering also the addition of each PGR alone with and without sugars as a carbon source.

4.3. Effect of Carbon Source and PGRs on Seedling Development

Results obtained in this work are consistent with those reported by several authors, who have indicated that high concentrations of soluble sugars have a negative effect on the development of organs (cotyledons, true leaves, roots) after germination [39]. Authors have indicated that low SUC concentrations inhibit the growth of *Brassica napus* hypocotyls and promote root growth probably by use as a source of carbon by counteracting their role in signaling [6]. Another study compared the effect of different carbon sources on the growth of Arabidopsis seedlings, indicating that 100 mM SUC and GLU-stimulated root growth 2-fold and increased rosette growth by almost 50% [39]. In this work, the effect of SUC and GLU on root growth was observed depending on whether CS was found to be high (1.0% and 2.3%) or low (3.2% and 5.0%), which directly influenced the average values of the different SVI treatments. Leaves' development was also different depending on the concentration of SUC in the medium. At low concentrations, the leaves were green, soft and changing from thin to round. At higher concentrations, the leaves had an abnormal pigmentation, presenting yellow/brown to dark green pigmentation, and showing small, thick leaves.

Several studies have indicated the positive effect of growth regulators on seedling growth. For example, shoot length, and root length in *D. purpurea* cultures increased up to 5.0 μM of cytokinins (BAP, Kin, TDZ) or auxins (AIA, NAA, and 2,4-D), whereas at 10 μM, these parameters were considerably lower even though germination was favored in the latter. However, the growth of the seedling was better than in a growth regulator free medium [40]. It has been pointed out that cytokinin requirements are extremely variable and depend on the endogenous content of each species [41]. On the other hand, the response of the tissues would be determined more by the hormonal proportion than by the individual levels of these [42]. Albacete et al. [43], in their review, indicated that auxins might have an antagonistic interaction with cytokinins during leaf initiation. Several reports indicate that cytokinins are necessary for shoot development and auxin for root growth, but their interaction is not synergic rather than competitive [40]. Our work agrees with the information provided by these authors, who reported that BA balanced seedling development by promoting shoot growth and slowing down root growth.

An increase in germination yield using different treatments can be a useful strategy, but the seedling vigor might be compromised. For example, SUC in the medium did not restrain the germination of *B. napus* but inhibited the hypocotyls growth and promoted root development [6]. This attribute cannot be neglected, because vigor of the seedlings is a critical aspect for the establishment of an *in vitro* culture. In this work, a slight increase in the capacity, time of germination and/or synchrony in *Taraxacum* seeds germination using NAA/BAP treatments was achieved, but seedling vigor was critically compromised under the proposed conditions due to low root development.

5. Conclusions

Carbon source concentration caused a higher impact factor on *T. officinale* germination, having a combined effect with PGRs treatments. Germination was promoted under the lowest carbon source concentrations (≤2.3%), whereas higher carbon source concentra-

tions (≥5.5%) had a detrimental effect on the germination process. GLU permits better germination capacity than SUC with higher FGP values, and uniformity/synchrony values seem to be enhanced by PGRs at combinations of 0.225–0.5 mg/L NAA and 0.5–1.25 mg/L BAP. As a result of this work, the best condition to assess *Taraxacum* seed germination was to use a medium containing GLU 2.3%, 0.5 mg/L NAA and 0.5 mg/L BAP. However, SUC accelerates germination, so the addition of this sugar to the culture medium should be considered.

Higher sugar concentrations (3.2–5.5%) and PGRs resulted in a less vigorous seedling compared with those that germinate at lower concentrations (1.0% and 2.3%). Therefore, two separate stages are recommended: an initial stage of germination in a medium containing GLU 2.3% as a carbon source and supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP, and the second stage of seedling growth in a medium containing SUC 2.3% with no PGR addition for seedling development—NAA and 1.25 mg/L BAP in combination. Rooting was best at SUC 2.3% when there were no PGRs in the medium.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7110486/s1, Table S1: Definition of Germination Measurement calculated in this work, Table S2: Main results of the MANOVA analysis of the tested variables on the germination of *T. officinale* seeds, Table S3: Main results of the MANOVA analysis of the tested variables on seedling development of *T. officinale* under the different CS and PGR conditions proposed, Table S4: Leaves number of *T. officinale* seedlings at different carbon source (glucose and sucrose) and Plant growth regulator (PGR) treatments, Table S5: Leaves length of *T. officinale* seedlings at different carbon source (glucose-GLU and sucrose-SUC) and Plant growth regulator (PGR) treatments, Table S6: Seedling vigour index (SVI) at different carbon source and plant growth regulators treatments.

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