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In Vivo Propagation of Root Explants of the Dandelion Species *Taraxacum kok-saghyz*, *Taraxacum officinale* and Their Interspecific Hybrids

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ABSTRACT

The dandelion species *Taraxacum kok-saghyz* is a promising new crop for natural rubber production and is currently in the early stages of the breeding process. To support these efforts, the multiplication and conservation of defined genotypes are crucial. While in vivo culture presents a practical method for this purpose, there is a lack of published data on its application to dandelion species, which needs to be addressed. In this study, a diverse set of 19 genotypes of *Taraxacum officinale*, *T. kok-saghyz* and their hybrids was propagated using root cuttings. Root explants were regenerated on Petri dishes and in Jiffy swell pots. Subsequently, plants were transferred to soil, and after a period of growth, biomass accumulation of root explants from different root sections, sprouting ability and survival rates were evaluated and compared to seed-grown plants. After 12 weeks, plants derived from in vivo explants produced lower root biomass than seed-grown plants, while shoot biomass remained unaffected. The ability to generate a rooted, cloned plant within just a few weeks represents a major advantage over the in vitro approach. *T. officinale* and its hybrids demonstrated higher survival rates than *T. kok-saghyz*. The study also demonstrated that both the root section used and the growth temperature influence plant regeneration. Additionally, the use of Jiffy swell pots further enhanced the regeneration rate. We successfully developed a simple and reproducible protocol for in vivo propagation to produce cloned genotypes. This method provides a cost-effective and rapid alternative to in vitro culture, which requires complex laboratory procedures.

1 | Introduction

Natural rubber (NR) is a vital resource for various industries and serves multiple purposes in agriculture, national defence, transportation, medicine and daily life (Wang et al. 2024; Yip and Cacioli 2002). While approximately 70% of all NR is used in tire production (Cornish 2017), it is also a fundamental raw material for the manufacturing of over 50,000 other products (Hodgson-Kratky et al. 2017). The significance of NR is highlighted through the fact that it was classified in the past

as critical raw material by the European Union until 2023 (<https://lgi.earth/blog-critical-raw-materials-act>, accessed 21 August 2025) and the United States (Critical Agriculture Materials Act of 1984). Synthetic rubber cannot replicate the properties of processed NR (Mooibroek and Cornish 2000). Thus, the rubber tree *Hevea brasiliensis* is so far the only commercially viable source of NR (Cornish 2001). Its plantations are predominantly located in tropical regions, where cultivation faces threats from social, economic and political factors (Luo et al. 2018), as well as diseases such as the South

Abbreviations: ANOVA, analysis of variance; DH, double haploid; IQR, interquartile range; n.s., not significant; NR, natural rubber; SALB, South American leaf blight; TKS, *Taraxacum kok-saghyz*; TO, *Taraxacum officinale*.

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American leaf blight (SALB) (van Beilen and Poirier 2007) and replacement by more profitable palm oil plantations (Dallinger 2011). The risk of declining NR availability stands in contrast to the increasing demand for this resource (<https://www.anrpc.org/news/anrpc-releases-monthly-nr-statistical-report%2C-november-2024>, accessed 14 March 2025). Consequently, there is an urgent need for alternative plants capable of addressing short-term NR shortages.

While over 2500 species can synthesize NR (Mooibroek and Cornish 2000), *Taraxacum kok-saghyz* (TKS) is one of the few that can produce NR of comparable quality to that of *H. brasiliensis* (Cornish and Xie 2015). TKS tolerates low soil quality, has a short life cycle and is adapted to the temperate climates of Europe and North America (Uteulin et al. 2023). Drought stress can be tolerated as well while producing high-quality rubber (Mofidi et al. 2024). This makes TKS a promising alternative for NR production. After TKS collections were made in 2008 in Kazakhstan (USDA-ARS accessions) (Hellier 2011), the first breeding programmes and projects were started, such as EU-PEARLS (GA No. 212872), DRIVE4EU (GA No. 613697), TARULIN (GA No. 0315971), TAKOWIND (GA No. 22002312), EVITA (GA No. 031A285A) or TARDISS (<https://news.osu.edu/nsf-funded-center-to-drive-development-of-us-natural-rubber-industry/>, accessed 10 April 2025). These past and ongoing projects have demonstrated the potential for continuously increasing NR content and yield, supporting the idea of transforming TKS into an agricultural crop. Recent reviews (2021) summarize progress in TKS breeding and corresponding biotechnology approaches (Kuluev et al. 2023; Salehi et al. 2021).

Depending on planting density and plant age, literature reports NR yields ranging from 10 to 464 kg/ha for wild TKS germplasm and first breeding lines (Arias et al. 2016; Bates et al. 2019; Eggert et al. 2018; King-Smith et al. 2025; Kreuzberger et al. 2016). Such NR yields cannot compete with those of *H. brasiliensis* yet, which are reported to range from 500 to 1500 kg/ha/year (van Beilen and Poirier 2007). Enhancing NR yields of TKS through increased NR content and root biomass is therefore essential. However, not only the root biomass needs to be considered in this context but also the upper-ground biomass to allow TKS to not be outcompeted by weeds in field cultivations. Thus, additional breeding goals focusing on various aspects of plant architecture for enhancing agronomic practicability need to be considered as well. Hybridization with closely related, robust-growing species presents a promising approach for developing a high-performing crop. This strategy allows for the utilization of regular or fixed heterosis and the introgression of advantageous genes or gene combinations from related species (Iaffaldano et al. 2018). Our study included hybrid plants to reflect the critical role of interspecific hybridization in breeding for high NR yield per hectare.

For hybridization efforts, species related to TKS are found within the genus *Taraxacum* F. H. Wigg. This extensive genus is comprised of roughly 60 sections and 3500 species, encompassing a wide range of reproductive methods (apomictic, autogamous and allogamous) and genetic variations (from diploid to polyploid representatives) (Kirschner and Štěpánek 1997; Kirschner

and Štěpánek 2008). This includes *T. officinale* (TO), which is widespread over Europe and North America. Many dandelions are in focus because of their traditional use as medical herb. Seed oil extract of TO was reported to have potential to act as antioxidant and inhibitor of certain tumour cells (Wu et al. 2025). However, TO should be seen in terms of taxonomy and nomenclature as the common name for the *Taraxacum* sect. *Ruderalia* Kirschner, H. Øllg. & Štěpánek, species complex that contains many different, often undescribed, species. Compared to more distant *Taraxacum* species, TO shows great potential of being a suitable crossing partner for TKS (Uhlemann and Thiele 2024) and should be taken into account for breeding process and this study as well.

Depending on the specific goals of a breeding programme, utilizing genetically homozygous or homogeneous plant material can offer substantial advantages for TKS and common dandelion breeding strategies. Homozygous plant material is characterized by having identical alleles for a given gene at both loci on homologous chromosomes (Flici 2022). Genetic uniformity significantly enhances the efficiency of gene and trait transmission because the genetic makeup passed on to offspring is predictable and consistent. Despite these advantages, the production of highly homozygous TKS plant material faces significant challenges due to its natural reproductive biology. TKS plants naturally prefer to cross-pollinate with other individuals, and a self-incompatibility mechanism prevents successful fertilization with their own pollen. However, the self-incompatibility mechanism can be broken by the mentor effect, which describes an overstimulation of the stigma with foreign pollen leading to self-pollination (Kirschner et al. 2013; Wollenweber et al. 2021). When attempts are made to force self-pollination over multiple generations, this in common leads to inbreeding depression. Repeated selfing increases the likelihood of individuals becoming homozygous for harmful recessive alleles, leading to non-viable offspring. Typically, such alleles are masked by dominant, functional alleles in a heterozygous state. Such biological challenges make it difficult to maintain an inbred line through successive generations of selfing.

Despite the biological challenges, the theoretical requirement to produce parental plants that are > 99% homozygous through selfing is indeed seven or more generations. This extended timeframe is a significant bottleneck in TKS breeding programmes. This challenge could be overcome by the generation of double-haploid (DH) homozygous plants by using microspores and chemically induced doubling of their haploid genome, instantly creating homozygous plants. While some DH protocols for members of the *Asteraceae*, such as sunflower (Mabuza et al. 2023), demonstrate the potential of this method, the processes for TKS are not yet well understood (Chandrasekera et al. 2017) and the problem of potential inbreeding depression due to accumulation of deleterious recessive alleles remains. For these reasons, the development of homozygous plant material still poses a lot of challenges in TKS breeding approaches, which leads to the application of homogeneity.

Genetically homogeneous parental plant material refers to material that has highly similar genetic characteristics, or is even genetically identical (e.g., a clone). However, clones may still be

highly heterozygous (in contrast to homozygous plant material). When such homogeneous, albeit heterozygous, parental plant material is used in different crossings, it can lead to similar and more consistent phenotypes for F1 offspring. For TKS, there are two main methods for developing homogenous plant material; in vitro and in vivo propagation.

In vitro propagation allows the generation of homogeneous clones and plant material in a controlled laboratory environment. This technique has already been examined for various species within the genus *Taraxacum*, including TO (Rawa'a et al. 2018), *Taraxacum platycarpum* (Lee et al. 2004), *Taraxacum brevicorniculatum* (Zhang et al. 2015), *Taraxacum belorussicum* (Gałuszka et al. 2019) as well as TKS (Cao et al. 2023; Yang et al. 2024). Notably, root explants and leaves exhibit a high regenerative potential. In vitro culture offers several advantages, including a high reproduction rate, assurance of plant purity and health and year-round propagation under defined laboratory conditions, independent of the natural environment (Hasnain et al. 2022; Lal et al. 2022). However, disadvantages include high laboratory costs and biotechnologically demanding procedures that pose a significant risk of contamination when sterile conditions are required. Additionally, in vitro cultivation carries the risk of losing the ability to grow under natural conditions, as well as the occurrence of somaclonal variations (Duta-Cornescu et al. 2023).

In vivo propagation, on the other hand, allows the multiplication and growth of cloned plants directly in their natural environment in soil. This method, which utilizes for example offshoots, cuttings, or tubers, is well-established for crops such as bananas, strawberries, potatoes and grapevines, facilitating their intensive agricultural use. Compared to in vitro propagation, in vivo propagation has the advantage of being a technically simple and more cost-effective method, which increases the gains of selection in breeding programmes by increasing the size of the base population for crossings. Because dandelion root explants possess a high regenerative capacity, they could be utilized for in vivo propagation. However, there is a lack of comprehensive data in the literature about the implementation and application of the in vivo propagation method for dandelion species.

Thus, the objectives of this study were to

- I. establish a simple protocol for in vivo propagation of root explants with high turnover rates under low-cost conditions for a diverse genotype set of the species TKS and TO as well as their interspecific hybrids;
- II. determine the optimal cultivation conditions for successful propagation of root explants; and
- III. compare the root architecture and above-ground biomass of plants raised from seeds and root explants with regard to the applicability of in vivo propagated plants for selection of yield and NR content in breeding programmes.

This study evaluates the advantages and limitations of in vivo propagation to assess its potential for the multiplication and conservation of dandelion genotypes.

2 | Materials and Methods

2.1 | Plant Material

Our study examined a diverse set of 19 genotypes, classified into three categories: TKS, TO and their interspecific hybrids (Table 1).

The TKS category comprised two USDA-ARS accessions TKS-35165 and TKS-35159 representing wild TKS material collected in Kazakhstan (Hellier 2011). Seeds for these accessions were obtained from the USDA-ARS National Germplasm System and selected due to the significant geographical separation of their collection sites (72 km). Additionally, we included intra-specific F1 hybrids from a cross between two TKS inbred lines (TK21×TK27) to represent genetically homogeneous material. The inbred lines were established through repeated self-fertilization over six generations, induced by pollination with mentor pollen from a true TO apomict. Progeny were verified by phenotype and PCR markers. A genotype from a breeding programme (TKS_NR) selected over several cycles for its high NR content (>7%) was also included to assess the influence of high NR content on in vivo propagation.

The TO category comprised seven genotypes selected to evaluate the potential effects of ploidy level and reproductive system on in vivo propagation. These included two diploids (DE3 and DW3b), four triploids (Kultivierter Vollherziger, Blühfreude, Nouvelle, B23-352) and one tetraploid (TO-E23). Regarding reproductive strategies, DE3 is autogamous; DW3b and TO-E23 are outcrossing; and all four triploids are apomictic. As autogamy and apomixis ensure high genetic homogeneity, these genotypes were selected to minimize the confounding effects of genetic background variability.

The hybrid category, consisting of eight genotypes, was subdivided into TO×TKS and TKS×TO groups to account for the influence of crossing direction on phenotypic traits (Uhlemann and Thiele 2024). The TO×TKS subcategory included two diploid outcrossing genotypes (DW2a×TKS, DW3b×TKS) and three triploid apomictic genotypes (BC2-H64, BC2-H80, BC2-H203) exhibiting strong TO characteristics. Conversely, the TKS×TO subcategory consisted of three diploid outcrossing genotypes (TKS×DW1a, TKS×DW2a, TKS×DW3b) with dominant TKS characteristics.

Seeds for the TO genotypes and interspecific hybrids were provided by ESKUSA GmbH (Parkstetten, Germany), the Julius Kühn-Institute in Quedlinburg (Institute for Biosafety in Plant Biotechnology, Germany) and Böhm Agrar GmbH & Co. KG (Kruckow, Germany). Voucher specimens for accession DE3 (HAL 163249) and DW3b (HAL 149940) are deposited at the Herbarium HAL, Institute for Biology, Department of Geobotany and Botanical Garden (Martin-Luther-Universität, Halle-Wittenberg). Ploidy levels of the accessions were determined via flow cytometry. Leaf material was chopped using a razor blade and added to 600 µL of CyStain nuclei extraction buffer (Sysmex Partec, Germany). Leaf pieces were removed from the solution by sieving through CellTrics Green devices (Sysmex Partec, Germany). The DNA in the extracted nuclei was stained with DAPI for fluorescence measurement by adding

TABLE 1 | Description of plant material used for the in vivo propagation method.

Species	Ploidy	Name genotype	Number plants	Reproduction
TKS	2n = 2X	USDA-ARS 35165	2	Outcrossing
		USDA-ARS 35159	6	
		TKS_NR	4	Outcrossing
		TK21×TK27	6	Outcrossing
TO	2n = 2X	DE3	4	Autogamous
		DW3b	5	Outcrossing
	2n = 3X	Kultivierter Vollherziger	5	Apomictic
		Blühfreude	4	
		Nouvelle	6	
		B23-352	84	
	2n = 4X	TO_E23	5	Outcrossing
Hybrid TO×TKS	2n = 2X	DW2a×TKS	6	Outcrossing
		DW3b×TKS	6	
	2n = 3X	BC2-H64	5	Apomictic
		BC2-H80	6	
		BC2-H203	6	
	Hybrid TKS×TO	2n = 2X	TKS×DW1a	6
TKS×DW2a			6	
TKS×DW3b			6	

200 µL CyStain staining solution (Sysmex Partec, Germany). For each sample, 1500 events were measured using Partec CyFlow Space, and the peak maximum was determined using Gaussian mode. The gain was selected for a diploid reference plant (chromosome count by microscopy) so that the peak maximum was approximately 180. Flow cytometry measurements for the genotypes are summarized in Table 1 and exemplarily shown in Supporting Information S1.

2.2 | Experimental Design and Preparation of Root Explants Under Temperate Growth Conditions

Seeds from 18 genotypes (Table 1, genotype B23-352 not included) were stored at −20°C and germinated at 20°C under high humidity. Two weeks post germination, plantlets were transferred into trays. After an additional 4 weeks, they were transferred again into pots (11 × 11 × 23 cm) at the Julius Kühn-Institute in Groß Lüsewitz (Institute for Breeding Research on Agricultural Crops; Germany; 54°07' N, 12°32' E). Plants were maintained under temperate greenhouse conditions (20–30 klx; day/night: 20°C/18°C, 16 h/8 h).

After 12 weeks of growth in soil, two to six plants per genotype (Table 1) were harvested. Shoots and roots were separated by cutting, and individual fresh weight was recorded. Roots were segmented into explants of approximately 1.5 cm in length and

≥ 0.1 cm in diameter; roots < 0.1 cm in diameter were discarded. The severed shoots, retaining approximately 1 cm of root, were replanted in soil to assess regrowth capability after 8 weeks. Additionally, two experimental setups were designed for the root explants:

Experimental Setup A: For each plant, one representative root explant from the upper/basal, middle and lower/apical section of the root was selected for in vivo propagation. In total, 279 root explants (from 93 plants) were randomly arranged in Petri dishes (12 × 12 cm) with filter paper under greenhouse conditions. Each Petri dish was divided into 24 equal squares to position the root explants. Upon leaf sprouting, explants were potted and cultivated for 12 weeks in a randomized greenhouse design, mirroring the conditions of the seed-raised plants. Subsequently, regenerated plants were harvested, and leaf and root weight measurements were recorded and compared with the 12-week-old seed-derived plants.

Experimental Setup B: All remaining root explants that were not used in Experimental Setup A were arranged in Petri dishes with moist filter paper, randomized per genotype. The time of sprouting was recorded for each root explant. After sprouting, the explants were transferred to trays with soil and cultivated for an additional 4 weeks. The sprouting percentage per genotype and the 4-week post-sprouting survival rate were calculated. Explants failing to sprout within 8 weeks

were discarded. Exemplary photos for the experimental setup and in vivo propagation method are visualized in Supporting Information S2.

2.3 | Optimization of Root Explant Regeneration With Jiffy Swell Pots

Seeds of the triploid, apomictic TO genotype B23-352 were sown in September 2023. In total, 84 plants were maintained in pots until the experiment commenced in February 2025. These plants were used to evaluate the effects of Jiffy swell pots on the regeneration of root explants. Plants were transferred from outdoor polytunnels (temperature: $\sim 1^{\circ}\text{C}$) to a greenhouse ($53^{\circ}54' \text{N}$, $13^{\circ}14' \text{E}$) maintained at 4°C at least 24 h prior to the experiment. On 17 February 2025, 1 day prior to the first sampling event, the greenhouse temperature was increased to 8°C .

Sampling occurred at three time points (18 February, 25 February and 5 March 2025) to assess the effects of cold temperature and acclimatization. Plants were carefully removed from the pots and roots were cleaned of the attached soil. Using a clean, sharp knife, 3-cm segments were excised from the basal, middle and apical sections of a long root. These explants were immediately planted in swollen Jiffy pots (Windhager HandelsgesmbH, Austria), maintaining the original root orientation.

Jiffy swell pots were arranged in trays filled with moist SERAMIS plant granulate. Explants from each one cutting region (basal, middle, apical) were allocated to separate trays. Trays were then randomized under plant lamps on a shelf with grids to ensure that the light reached the bottom shelf. Thus, nested randomization was ensured. Substrate moisture was maintained constantly. Mean temperature was 19°C and the average air humidity was 55%. Development of the root cuttings was scored twice a week in comparison to a phenotyping scale (Supporting Information S3), starting 1 week after root sectioning. The phenotyping scale ranged from Stadium 1 representing undifferentiated cell clusters to Stadium 9 with big leaves.

For statistical analysis, data collected up to 37 days post-sampling were included. To ensure orthogonal data, all genotypes in which one or more root cuttings had not sprouted or had died were excluded.

2.4 | Data Evaluation

To assess the effects of genotype on root explant regeneration and the impact of in vivo propagation on root architecture, means and standard deviations were calculated for individual genotypes and genotype groups. Outliers were excluded using the $1.5 \times \text{IQR}$ rule. Statistical comparisons were performed using one- and two-way analyses of variance (ANOVAs) and two-sided *t*-tests. All analyses were conducted in RStudio (version 2024.12.0) using the car package (Fox and Weisberg 2019). For graphical illustration of the results, the package ggplot2 v3.3.3 (Wickham 2011) was used.

3 | Results

3.1 | Shoot and Root Weight of 12-Week-Old Plants Grown From Seeds

Plants grown from seeds were analysed after 12 weeks and categorized into different genotype groups by ploidy: TKS $2X = 2n$ (TKS_2X), TO $2X = 2n$ (TO_2X), $3X = 2n$ (TO_3X), $4X = 2n$ (TO_4X) and hybrids $2X = 2n/3X = 2n$. Hybrids were further distinguished by crossing direction (TKS $\times$ TO_2X, TO $\times$ TKS_2X, TO $\times$ TKS_3X). Measurements of root and shoot weight (Table 2, Figure 1) revealed significant ($\alpha = 0.05$) differences between TKS and all other groups for root (Supporting Information S4) and shoot weight (Supporting Information S5), where root and shoot weight measurements were two to three times lower for the TKS group compared to the others. No significant difference ($\alpha = 0.05$) in root weight was observed within TO and hybrid groups or between these two groups for shoot weight. TO_3X genotype showed significantly ($\alpha = 0.05$) higher values in comparison to TO_4X and TO $\times$ TKS_3X. When considering single genotypes, the highest root and shoot weight measurements were for TO_Blühhfreude (root: 16.41 g, shoot: 35.76 g) and the lowest for TKS-35159 (root: 1.89 g) as well as TK21 $\times$ TK27 (shoot: 5.26 g) (Supporting Information S6).

3.2 | Regeneration of Decapitated Plants

Decapitated shoots with 1-cm remaining root were placed on soil to assess regrowth potential. A total of 93 plants across TKS, TO and hybrid groups were evaluated. More than 90% of plants shed all leaves within a few days. Notably, two plants flowered within a week after decapitation but failed to set seed. After 8 weeks, regeneration was observed in 20/30 (66.7%) of TO, 19/43 (44.2%) of hybrids and 5/18 (27.8%) of TKS plants. When looking at single genotypes, especially TKS and TKS $\times$ TO hybrids, were forming no new roots in some cases, but were still alive and forming small leaves (< 1 cm length). For TO and TO $\times$ TKS hybrids, new roots were formed more frequently together with leaves compared to TKS (Supporting Information S7).

3.3 | Influence of Root Section on Survival Rate of In Vivo Propagated Plants

To assess whether root diameter and section position influence sprouting ability in root explants from 12-week-old plants under moderate temperatures, roots were divided in Experimental Setup A into three sections—upper (basal), middle and lower (apical). Explants from each section were placed on Petri dishes, and their sprouting ability was monitored over an 8-week period. Comparing all genotypes together, the results showed a progressive decrease in sprouting ability from the basal to the apical end (Figure 2). After 8 weeks, 83.0% of basal explants, 66.0% of middle explants and 57.4% of apical explants had sprouted.

Following sprouting, the root explants were transferred to pots and grown for an additional 12 weeks. Notably, 63.8% of basal,

TABLE 2 | Root and shoot weight for different genotype groups after 12 weeks of growth on soil for plants cultivated from seeds; outliers were excluded using the 1.5×IQR rule.

Genotype	Grown from seeds			Grown from root explants		
	Mean root weight (g) ± SD	Mean shoot weight (g) ± SD	Plants for analysis	Mean root weight (g) ± SD	Mean shoot weight (g) ± SD	Plants for analysis
TKS, 2X=2n	2.75 ± 1.33	6.50 ± 2.67	16	0.64 ± 0.64	3.52 ± 5.06	16
TO, 2X=2n	6.65 ± 3.46	24.28 ± 3.41	10	2.33 ± 2.06	19.62 ± 17.46	16
TO, 3X=2n	9.62 ± 3.86	28.09 ± 5.65	14	5.04 ± 4.24	28.71 ± 23.10	26
TO, 4X=2n	6.62 ± 3.07	14.10 ± 1.94	6	3.53 ± 1.68	26.64 ± 14.01	11
TO×TKS, 2X=2n	8.22 ± 2.24	22.50 ± 4.39	12	4.04 ± 2.59	23.07 ± 16.69	24
TKS×TO, 2X=2n	8.81 ± 5.53	22.00 ± 13.05	15	3.15 ± 2.11	16.89 ± 10.55	26
TO×TKS, 3X=2n	7.87 ± 4.50	15.69 ± 5.52	16	5.34 ± 3.58	24.46 ± 16.98	26
TKS, all ploidy	2.75 ± 1.33	6.50 ± 2.67	16	0.64 ± 0.64	3.52 ± 5.06	16
TO, all ploidy	8.02 ± 3.87	24.23 ± 6.79	30	3.91 ± 3.48	25.52 ± 20.18	53
Hybrid, all ploidy	8.30 ± 4.45	19.61 ± 9.12	43	4.18 ± 2.98	21.37 ± 15.31	76

55.3% of middle and 44.7% of apical root explants formed new plants and survived the full 12 weeks. At the end of this period, root and shoot biomass were measured and compared to those of seed-grown genotypes (Table 3). All genotype groups grown from seeds had significantly higher root biomass compared to those grown from root explants (Figure 3A, Table 3), with the exception of the genotype group TO×TKS_3X. Group TO_4X showed the same trend, but the differences were not statistically significant (*p* value 0.056). However, although biomass was lower, the roots of explant-grown plants exhibited a thinner diameter.

Regarding shoot biomass, no genotype group showed significant differentiation between plants grown from seeds and in vivo propagation while TKS_2X and TO×TKS_3X were close to being statistically significant (Figure 3B, Table 3). A comparison of the mean root and shoot weight showed for all 18 genotypes that plants grown from seeds exhibit a stronger growth than plants grown from root explants after 12 weeks (Supporting Information S8 and S9).

When examining root and shoot biomass across the genotype groups TKS, TO and hybrid, as well as the seed and in vivo propagation method, clear differences emerge (Figure 4). For all three genotype groups, seed-grown plants show stronger root growth than plants regenerated from in vivo explants, in some cases significantly so. Among the in vivo explants, root growth declines from basal to apical sections, a decrease that is significant for TO and hybrid genotypes (Figure 4A). In contrast, no comparably clear trend is observed for shoot growth. Significant differences were determined, but occur together with large error bars and small sample sizes (Figure 4B).

3.4 | Sprouting Ability, Survival Rate and Ranking of Genotypes

In Experimental Setup B, we examined all remaining root explants that were not included in Experimental Setup A. The results revealed that the sprouting ability of root explants increased during the first 2 weeks on Petri dishes, followed by a steady decline after 4 weeks (Figure 5). However, sprouting continued even after 8 weeks. Over time, the growth of algae and fungi was observed on all Petri dishes. In total, 2708 root explants were placed on Petri dishes, of which 1060 (39.1%) sprouted after 8 weeks. Among genotypes, the TKS category exhibited the highest sprouting ability with about 45.1%, followed by TO with 40.0% and interspecific hybrids with 37.5%. Sprouting abilities for genotypes ranged from 32.3% for TO×TKS_3X, 37.0% for TKS×TO_2X, 38.4% for TO_2X, 40.0% for TO_3X, 45.1% for TKS_2X, 45.5% for TO_4X to 52.4% for TO×TKS_2X. The number of sprouted root explants per plant was highest for hybrids (13.3), followed by TO (10.5) and TKS (8.1). The maximum number of sprouting root explants for one plant was 42 for both TO and hybrids and 23 for TKS.

All sprouted root explants were transferred to soil, and the survival rate was documented after 4 weeks. The survival rate of sprouted explants was highest for hybrids (91.4%), followed by TO (90.6%) and TKS (79.5%).

To assess the stability of root and shoot weight across seed and in vivo propagation methods, a genotype ranking was calculated (Supporting Information S10). The analysis revealed that the propagation method did not influence the ranking of TKS, TO and hybrid genotypes, indicating consistent performance across propagation techniques.

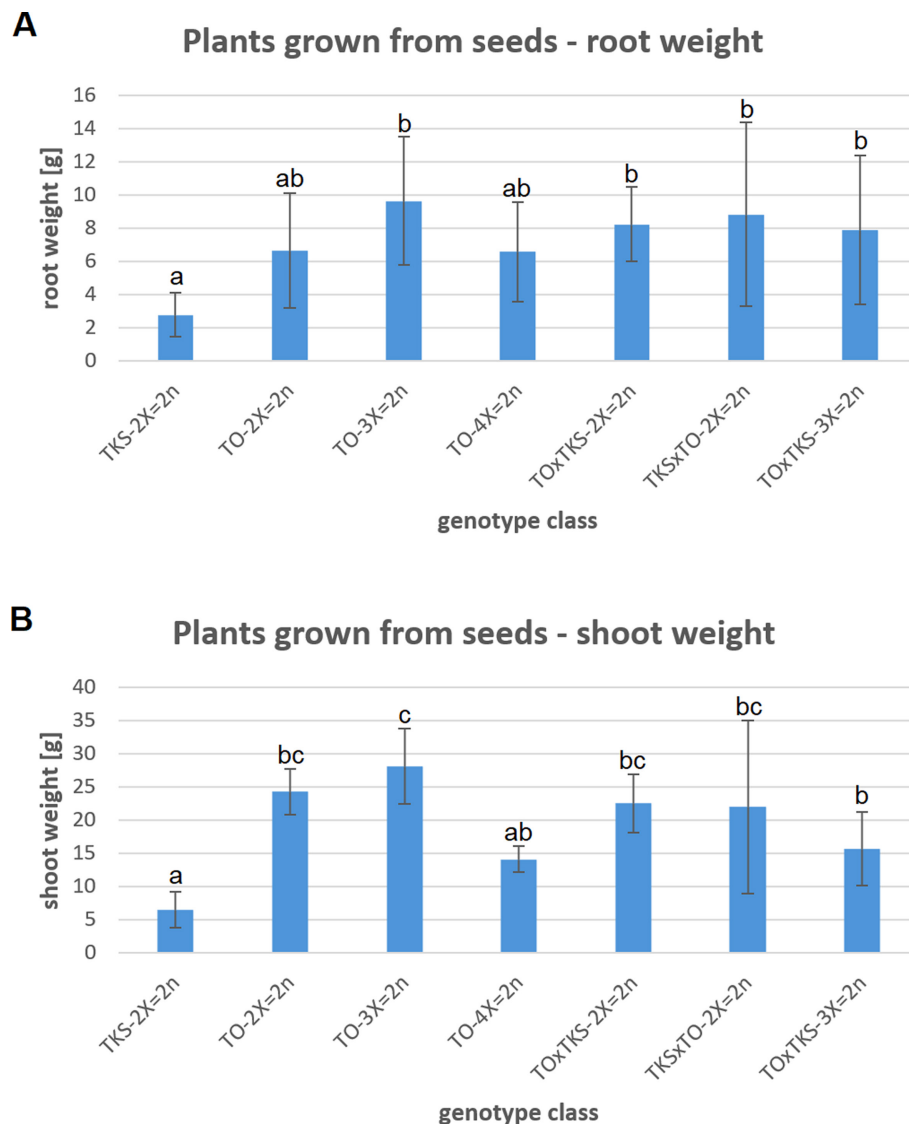


FIGURE 1 | Root and shoot weight for different genotype groups after 12 weeks of growth on soil for plants cultivated from seeds; A = root weight, B = shoot weight. Means followed by a common letter are not significantly different according to the Tukey test. Number of plants used for analysis: TKS-2X=2n: 16, TO-2X=2n: 10, TO-3X=2n: 14, TO-4X=2n: 6, TOxTKS-2X=2n: 12, TKSxTO-2X=2n: 15, TOxTKS-3X=2n: 16; outliers were excluded using the 1.5×IQR rule. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

Sprouting of root explants on Petri dishes regarding root section

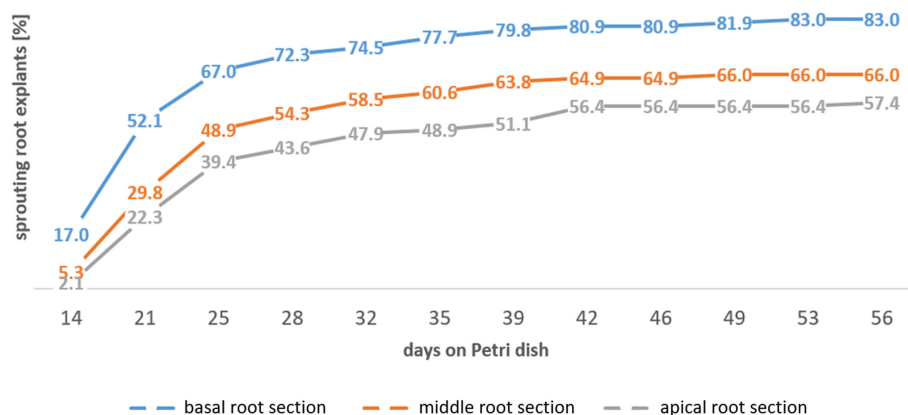


FIGURE 2 | Sprouting roots over time for the in vivo propagation method examining basal to apical root sections; blue line = upper root section (basal), orange line = middle root section, grey line = lower root section (apical). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

TABLE 3 | Significance level of contrasting root and shoot weight for plants raised from seeds and root explants after 12 weeks of growth.

Genotype	Root		Shoot	
	<i>p</i>	Significance level	<i>p</i>	Significance level
Hybrids	2.48e−08	***	0.5092673	n.s.
TKS	4.7e−06	***	0.0555428	n.s.
TO	4.8e−06	***	0.7515278	n.s.
TKS, 2X=2n	4.7e−06	***	0.0555428	n.s.
TO, 2X=2n	0.0007917	***	0.4544388	n.s.
TO, 3X=2n	0.0022416	**	0.9265461	n.s.
TO, 4X=2n	0.024869	*	0.0881287	n.s.
TKS×TO, 2X=2n	5.11e−05	***	0.2043423	n.s.
TO×TKS, 2X=2n	5.24e−05	***	0.9142284	n.s.
TO×TKS, 3X=2n	0.055864	n.s.	0.0578428	n.s.

Note: Outliers were excluded using the 1.5×IQR rule.

n.s. (not significant): $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.

3.5 | Impact of Jiffy Swell Pots and Low Temperatures on In Vivo Propagation

Overall, root explants of genotype B23-352 cultivated in Jiffy swell pots exhibited regeneration and survival rates of 70% for basal, 80% for middle and 85% for apical segments (Table 4). As mentioned above, 18 genotypes on Petri dishes resulted in lower average survival rates: 63.8% for basal, 55.3% for middle and 44.7% for apical explants. Notably, apical segments achieved the highest survival rates in Jiffy swell pots, whereas basal segments performed best in Petri dishes. Furthermore, it was observed that the date of harvest and different time spans of acclimatization to moderate temperatures influenced plant regeneration. Later harvest dates for root explants from all root sections corresponded with significantly higher plant regeneration rates (Table 4, Supporting Information S11 and S12). Phenotypic evaluation confirmed that later harvest dates combined with an extended acclimatization period significantly improved plant regeneration (Figure 6).

4 | Discussion

Different regeneration systems have been successfully studied and applied across various *Taraxacum* species. Direct organogenesis involves the direct development of organs from meristematic cells, whereas indirect organogenesis includes an intermediate callus phase (Verbitskaya et al. 2021). Both approaches can result in the formation of plant structures such as shoots, roots, leaves, or flowers. In vitro culture techniques typically utilize indirect organogenesis to produce pathogen-free plants year-round with high multiplication rates. However, this method poses significant challenges, as its success depends on numerous factors, including medium composition (Verbitskaya et al. 2021), the type and combination of growth regulators (Martínez et al. 2021), light conditions (Blair Nameth et al. 2018), sucrose concentration (Gałuszka et al. 2019) and temperature (Serivichyaswat et al. 2022). Despite its benefits,

in vitro cultivation is associated with high laboratory costs and a considerable risk of contamination. These drawbacks have led to increased interest in in vivo propagation methods as an alternative for *Taraxacum* species.

4.1 | Comparison of In Vitro and In Vivo Propagation Methods

The primary advantages of in vivo propagation include low consumable costs, minimal space requirements (e.g., for Petri dishes or Jiffy pots) and the elimination of the need for a sterile laboratory. In vivo methods propagate plants with initial leaves within 4 weeks, compared to the minimum of 3 months typically required for in vitro propagation (personal communication). This rapid preservation and multiplication of target genotypes has the potential to increase the efficiency of breeding programmes.

Crosses performed using in vivo propagated and multiplied inbred lines—and thus identical plant material—generate genetically homogeneous progeny that possess the desired genetic background and more consistent phenotypes. Furthermore, this approach facilitates the mass propagation of elite selections, allowing specific genotypes to be included more extensively in breeding programmes. Although crossing barriers exist between different dandelion subspecies (Uhlemann and Thiele 2024), clonal multiplication accelerates the identification of valuable crossing partners by enabling simultaneous experimental crosses. Additionally, it increases seed output for specific crossings by allowing those crossings to be replicated out multiple times.

While both in vitro and in vivo methods can deliver plants with reduced infection pressure and limit disease spread, the risk of contamination needs to be considered for both methods. Algae and fungi appeared in Petri dishes with filter paper after 4 weeks. However, since most root explants

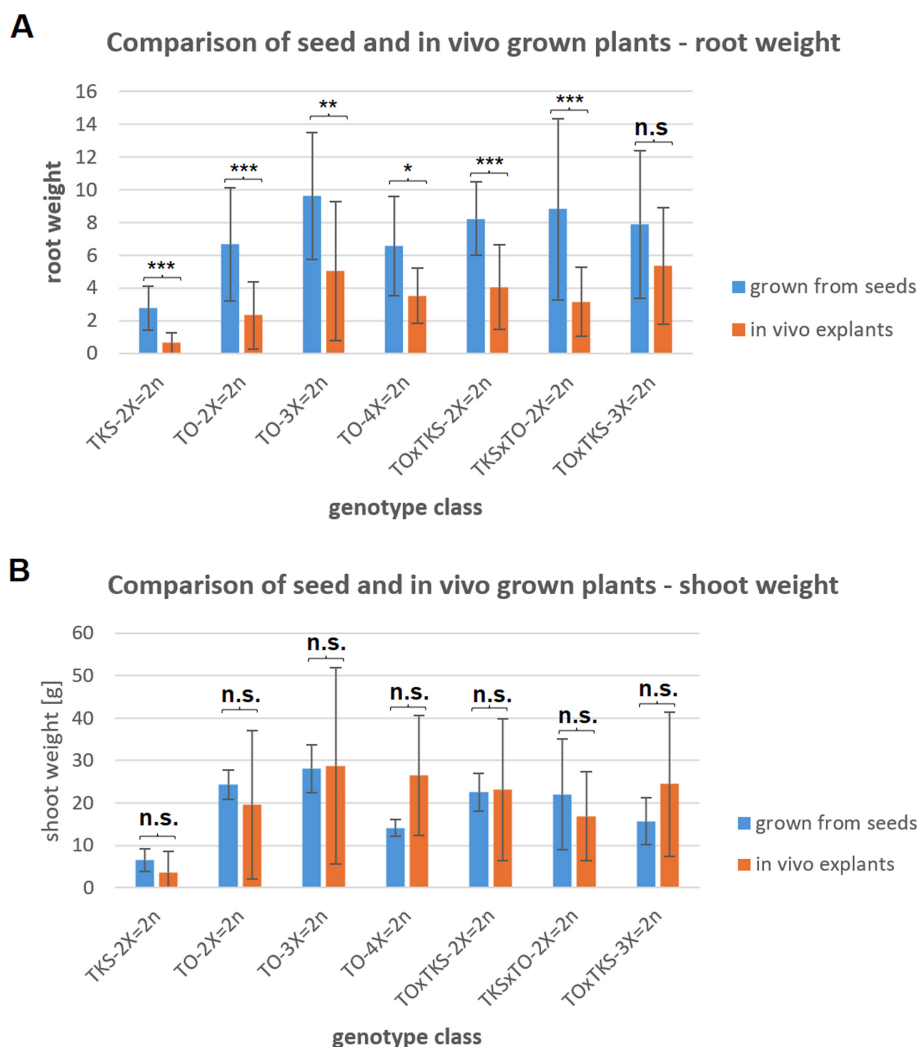


FIGURE 3 | Comparison of root and shoot weight for different genotype groups including plants raised from seeds and root explants after 12 weeks of growth. A = root weight, B = shoot weight, blue columns = plants raised from seeds, red columns = root explants raised with the in vivo propagation method. Number of plants used for analysis: TKS-2X = 2n, seed: 16, in vivo: 16; TO-2X = 2n, seed: 10, in vivo: 16; TO-3X = 2n, seed: 14, in vivo: 26; TO-4X = 2n, seed: 6, in vivo: 11; TOxTKS-2X = 2n, seed: 12, in vivo: 24; TKSxTO-2X = 2n, seed: 15, in vivo: 26; TOxTKS-3X = 2n, seed: 16, in vivo: 26; outliers were excluded using the $1.5 \times \text{IQR}$ rule. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

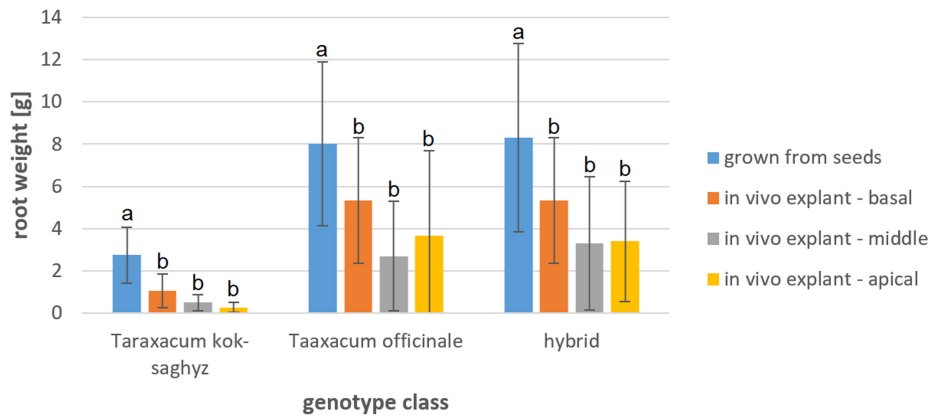
began sprouting within 2–4 weeks (Figure 5), this contamination issue may be considered negligible. Transferring the root explants to Jiffy swell pots effectively eliminated fungal contamination. Although algae were still visible on the exterior of the Jiffy swell pots, they did not inhibit root growth. Furthermore, Jiffy swell pots offer additional benefits: previous studies indicate that they facilitate plant acclimatization, promote root growth and nutrient uptake, and reduce plant loss (Neri et al. 2017; Peixe et al. 2015).

While in vivo propagation of dandelion species utilizes root material, in vitro culture typically relies on leaf blade cuttings for regeneration (personal communication). Potential multiplication rates vary by genotype and depend on the availability of leaves and roots as plant material. Because of the fast regeneration of leaves, in vitro culture guarantees high multiplication rates without limitation. However, using the in vivo propagation method, it was possible to regenerate up to 42 clones from one single plant within 1 month.

For in vivo propagated plants, we demonstrated that after 12 weeks of regeneration (Experimental Setup A), root and shoot weight measurements differed between root explants of different root sections. Clones derived from Petri dishes of basal root segments exhibited significantly higher root and shoot weight measurements, whereas those from apical root segments showed the lowest weight (Figure 4). Consequently, the uniformity of regenerated plants regarding biomass accumulation within the first 12 weeks of growth was limited. To mitigate these experimental limitations, explants should be consistently derived from a single, specific root section. While it is plausible that disparities in shoot and root weight will converge over extended growth periods, this hypothesis requires confirmation through future studies.

It is also worth noting that while somaclonal variations and DNA methylation are often cited as reasons for phenotypic variation (Ghosh et al. 2021), they were not examined here. Consequently, differences in genotype and root diameter were

A Comparison of seed and in vivo grown plants - root weight



B Comparison of seed and in vivo grown plants - shoot weight

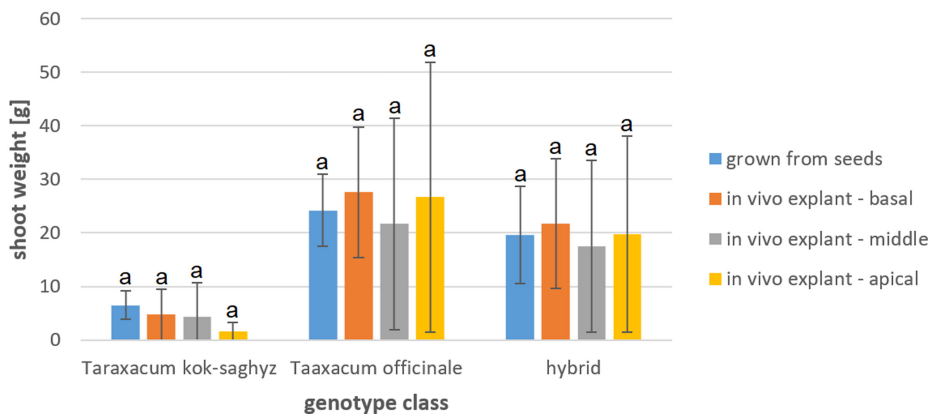


FIGURE 4 | Root and shoot weight after 12 weeks of growth for plants grown from seeds and in vivo propagation explants from basal, middle and apical root sections; A = root weight, B = shoot weight, blue column = plants grown from seeds, orange column = in vivo propagation explants from the basal root section, grey column = in vivo propagation explants from the middle root section, yellow column = in vivo propagation explants from the apical root section. Means followed by a common letter are not significantly different according to the Tukey test. Number of plants used for analysis: *Taraxacum kok-saghyz*, seed: 17, basal: 6, middle: 5, apical: 5; *Taraxacum officinale*, seed: 31, basal: 17, middle: 16, apical: 20; hybrid, seed: 44, basal: 32, middle: 28, apical: 16; outliers were excluded using the $1.5 \times \text{IQR}$ rule. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

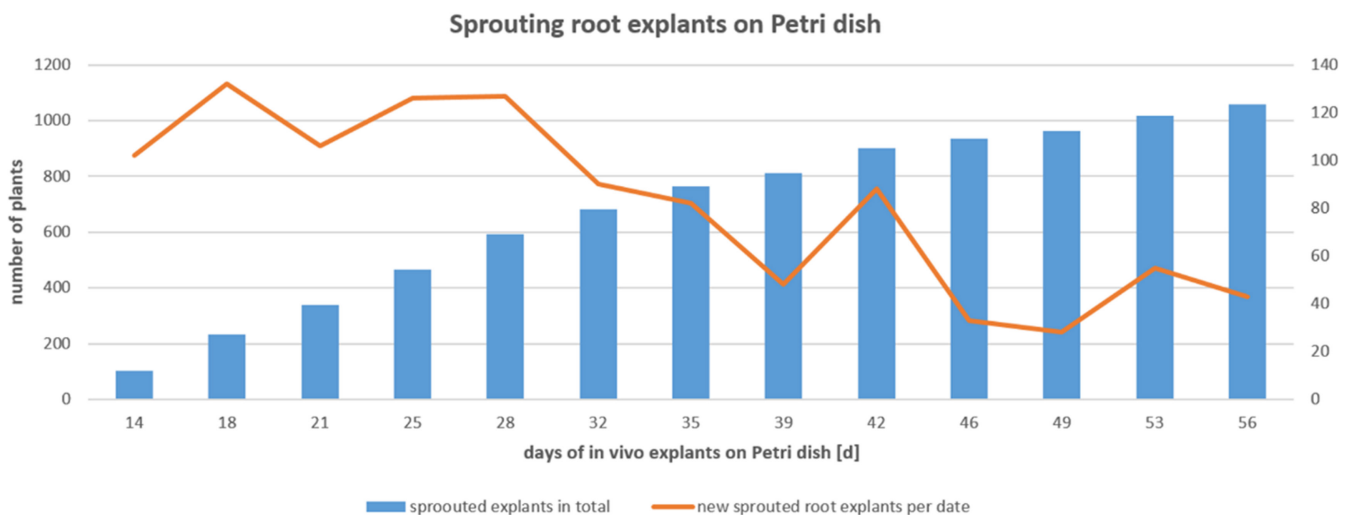


FIGURE 5 | Sprouting root over time for the in vivo propagation method for Experimental Setup B with all remaining root explants. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

TABLE 4 | Survival rate of root explants grown in Jiffy swell pots.

Phenotyping date	Basal root explants		Middle root explants		Apical root explants		All root sections	
	Survival rate	Plants for analysis	Survival rate	Plants for analysis	Survival rate	Plants for analysis	Survival rate	Plants for analysis
18.02.2025	75.0%	28	85.7%	28	82.1%	28	70.2%	84
25.02.2025	64.3%	27	67.9%	27	71.4%	27	79.8%	81
04.03.2025	71.4%	28	85.7%	28	82.1%	28	84.5%	84

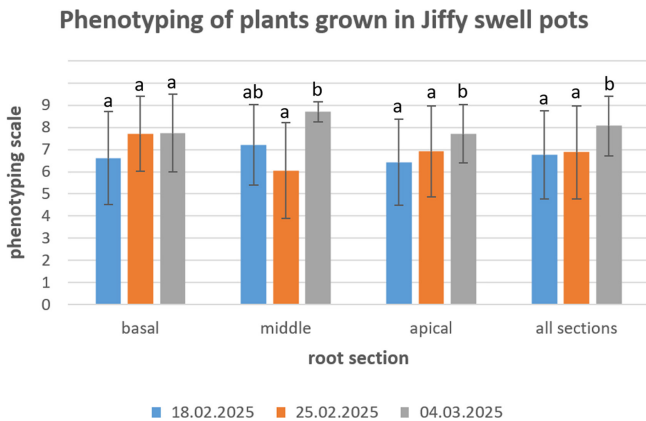


FIGURE 6 | Phenotyping of in vivo plant regeneration of root explants that were grown in Jiffy swell pots, blue column = root explants from basal root section, orange column = root explants from middle root sections, grey column = root explants from apical root sections. Number of plants used for analysis: 18 February 2025, basal: 21, middle: 24, apical: 23, all sections: 68; 25 February 2025, basal: 18, middle: 19, apical: 23, all sections: 60; 4 March 2025, basal: 20, middle: 24, apical: 25, all sections: 69. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

identified as the most likely causes of the observed phenotypic variation.

4.2 | Comparison of Plants Cultivated From Seeds and In Vivo Explants From Petri Dishes

A comparison of biomass within genotype groups propagated from seeds and the in vivo method showed that all groups, except TO×TKS_3X, exhibited significantly higher root weight after 12 weeks of growth when propagated from seeds compared to in vivo (Figure 3A, Table 3). These significant differences in root biomass did not appear to substantially affect above-ground biomass. No group showed a notable deviation for shoot biomass when comparing seed and in vivo propagated plants (Figure 3B, Table 3). We observed that plants derived from root explants produced fewer lateral roots during early developmental stages compared to seed-grown plants. This reduced lateral root formation may have restricted nutrient and water uptake, potentially limiting root growth and biomass accumulation. Since the experiment was limited to 12 weeks, it remains uncertain whether these biomass differences would persist or equalize over a longer growing period.

In experimental setup A, root weight accumulation decreased progressively from the basal to the middle and apical sections across all genotypes (Figure 4A). Variations in biomass accumulation may have different reasons. One potential explanation is that basal root fragments had a larger diameter than apical segments. Thicker roots generally possess greater elongation potential than thinner ones, resulting in higher biomass (Lecompte and Pagès 2007). Additionally, hypocotyl and stolon roots undergo secondary thickening—a process that is less pronounced in apical roots (Ragni and Greb 2018). Consequently, apical root explants may have a lower capacity for rapid biomass accumulation compared to basal root explants. These factors likely explain the observation that basal root explants tend to yield greater root biomass.

Dandelion seed germination varies based on factors such as population genetics, seed age and storage conditions like temperature and humidity. Freezing seeds extends viability and reduces germination time (Martinková and Honěk 2007). Under optimal conditions, germination rates often exceed 75% (Keener et al. 2018), with minimal subsequent plant loss. Sprouting rates for root explants from experimental setup A were slightly lower: 66.6% for TKS, 74.2% for TO and 65.9% for hybrids.

In experimental setup B, survival rates after 8 weeks were 91.4% for hybrids, 90.6% for TO and 79.5% for TKS. Thus, both seed and in vivo propagation methods demonstrate high survival rates once the plants are established. However, TKS plants from root explants exhibited the highest mortality rates. Potential causes—such as genetic background, root-specific differences, or chemical composition—require further research. Notably, the sprouting ability of root explants was significantly lower in Experimental Setup B compared to Setup A. Setup B contained all remaining root explants, including those from the lower sections of the roots. It is likely that this setup contained a higher proportion of explants unsuitable for in vivo propagation (e.g., small root diameter), which may have contributed to the reduced sprouting ability.

A ranking of genotypes often provides important information for breeding programmes and helps to evaluate and integrate trait stability and performance of genotypes across different environments. It was shown that the method of propagation (seed vs. in vivo) and the traits of root and shoot weight did not correlate significantly (Supporting Information S10), and the propagation method did not influence the ranking of TKS, TO and hybrid genotypes. Therefore, these genotypes demonstrate

stable relative performances regardless of the propagation technique. This stability facilitates the assessment of phenotypic traits, suggesting that both seed and in vivo methods can be used effectively while maintaining high phenotypic stability for root and shoot weight.

4.3 | Impact of Genotypes and Growth Conditions on the In Vivo Propagation Method

The USDA-ARS accessions TKS-35165 and TKS-35159 were collected approximately 70 km apart, a substantial geographical distance for TKS USDA-ARS collection sites. Despite this, they exhibit low genetic differentiation based on molecular genetic profiles (Kaiser et al. 2025). However, they showed clear differences in biomass accumulation and sprouting ability (Supporting Information S8). These findings suggested that genotype can significantly influence the effectiveness of in vivo propagation methods.

Measurements of root weight, sprouting and survival rates for TKS accessions revealed that the accession TKS_NR (high NR content of >7%) was capable of regenerating plants from root cuttings as effectively as accessions with lower NR content (Supporting Information S8). However, due to the small sample size and limited number of genotypes, we could not determine a clear positive or negative effect of NR content on sprouting ability. This remains a subject for potential further investigation. In addition to NR content, our experimental setup also allowed us to assess the influence of the ploidy level. We observed within the TO genotype group the highest sprouting ability for tetraploid and the lowest for diploid accessions. However, within TO × TKS hybrids, the trend of sprouting ability was with 52.4% for diploid and 32.3% for triploid accessions the opposite. Thus, it remains unclear if the ploidy level systematically influences sprouting ability.

To assess the impact of temperature on root explant regeneration after root cutting, accession B23-352 plants were initially kept in cold tunnels and later acclimatized under moderate greenhouse conditions. Root explants from the third phenotyping date (04.03.2025), which underwent a longer acclimatization period, subsequently exhibited significantly higher regeneration rates and stronger plant growth (Table 4, Figure 6). Several factors may explain these results. Elevated temperatures enhance metabolic activity and auxin biosynthesis, which promote root elongation (Fei et al. 2019). Additionally, suboptimal soil temperatures can reduce the colonization and activity of arbuscular mycorrhizae in various plant species; these fungi play a crucial role in nutrient uptake and root development (Liu et al. 2004), a mechanism that may also apply for dandelion species. Furthermore, low temperatures have been shown to inhibit root growth in various crops (Friero et al. 2023), suggesting that moderate temperatures are necessary to maximize success rates for in vivo propagation. Regeneration rate and plant growth were assessed using a phenotyping scale (Supporting Information S3). While this scale simplified the experimental setup and facilitated the drawing of several conclusions, it is inherently subjective and dependent on the individual performing the phenotyping. To ensure a more objective evaluation of regeneration rate and

plant growth, quantification of morphological parameters—such as leaf length, leaf width, leaf area, number of rosette leaves and canopy diameter—should be considered for future experiments.

In our study, Jiffy swell pots had a positive effect on root explant regeneration compared to root cuttings regenerated on Petri dishes. While explants derived from basal root sections showed only minor differences between methods, significantly higher regeneration rates were recorded for explants from the middle and apical root sections when using Jiffy pots. Specifically, explants on Petri dishes showed decreasing sprouting ability towards the apical root sections, whereas those in Jiffy swell pots exhibited the highest sprouting ability for these segments. This observation may be attributed to several factors. Basal root sections have a larger diameter and may be more resistant to the suboptimal conditions of Petri dishes, whereas the smaller diameter of apical explants may reduce their viability in that environment. The enhanced sprouting ability of apical root sections in Jiffy pots might be explained by the higher concentration of meristematic cells in root tips compared to basal sections (Perilli et al. 2012), which could account for higher sprouting rates when conditions are favourable. Furthermore, Jiffy swell pots ensure a sufficient supply of water and nutrients, supporting the establishment of apical explants that otherwise failed to survive on Petri dishes.

The aim of this work was to establish a simple protocol for the in vivo propagation method and to describe the fundamental differences between TKS, TO and their hybrids. By including a variety of different genotypes, we provided a proof of concept for this method. However, it is important to note that the sample sizes for individual genotypes were small. For seed-propagated plants, only two to six individuals per genotype were analysed, and for some in vivo-propagated genotypes (e.g., TKS_NR and TK21 × TK27), only five plants were available. Furthermore, analysed wild plant material (e.g., for TKS) possess a substantial heterogeneity, which also might influence the results. The large error bars in Figures 1, 3 and 4 likely reflect these limitations. Among plants regenerated through in vivo propagation, regeneration capacity and biomass growth varied considerably within genotypes. Now that the method has been successfully established, future work should prioritize larger sample sizes to better characterize differences between genotypes and ensure more robust statistical analyses.

5 | Conclusion

TKS produces high-quality NR in its roots, making it a promising alternative resource to meet the growing global demand for NR. To develop TKS from a wild species into a high-performing crop, targeted breeding programmes are essential. These efforts rely on the efficient multiplication and conservation of desirable genotypes, which is often achieved through in vitro culture that involves complex laboratory techniques.

This study presents a simple, low-cost protocol for the in vivo propagation of dandelion species using root explants, offering a practical alternative to in vitro methods. This protocol was

successfully applied to a diverse set of 19 genotypes, including TKS, TO and their hybrids. In vivo plant regeneration times were shown to be faster than those achieved using conventional in vitro techniques. Importantly, the propagation method did not affect the relative ranking of TKS, TO and hybrid genotypes with respect to root growth, indicating consistent performance across in vivo and seed-based propagation methods. Planting root cuttings in Jiffy swell pots enhanced plant regeneration and, consequently, increased the overall multiplication rate per plant, particularly for explants derived from apical root sections. Temperature was identified as a critical factor influencing the success of the in vivo propagation process. Future studies might investigate the effects of additional environmental factors, such as light intensity and humidity, to further optimize the in vivo propagation method.

The preservation and multiplication of target genotypes through in vivo propagation have the potential to improve the efficiency of breeding programmes. This approach enables the generation of genotypes with desirable genetic backgrounds and more uniform phenotypes, while also facilitating the mass propagation of selected genotypes for broader inclusion in breeding schemes. Furthermore, it supports increased seed production for targeted crosses, representing a crucial step towards accelerating breeding progress.

Author Contributions

René Kaiser: conceptualization, data curation, formal analysis, investigation, project administration, writing – original draft, writing – review and editing. **Britta Öhrlein:** conceptualization, data curation, investigation, writing – original draft. **Nicole van Deenen:** investigation, review and editing. **Helge Fließ:** writing – original draft, writing – review and editing. **Axel Schechert:** conceptualization, formal analysis.

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Ethics Statement

This study on plants, including the collection of plant material, complied with relevant institutional, national and international guidelines and legislation. All sources were legally acquired in accordance with the Nagoya Protocol.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** Shows representative flow cytometry measurements for the genotypes TO (2X = 2n), TKS (2X = 2n), hybrids TKS × TO (2X = 2n) and TO (3X = 2n). **Data S2:** Illustrates key steps of the in vivo propagation method. **Data S3:** Provides a phenotyping scale for the development of root explants cultivated in Jiffy swell pots. **Data S4:** Presents the statistical significance of differences in root weight among genotype classes grown from seed for 12 weeks. **Data S5:** Presents the statistical significance of differences in shoot weight among genotype classes grown from seed for 12 weeks. **Data S6:** Provides raw data for root and shoot weight measurements of genotypes grown from seed for 12 weeks on soil. **Data S7:** Presents the capacity of decapitated shoots to form new leaves and roots after 8 weeks of regeneration on soil across all genotypes. **Data S8:** Provides root and shoot weight data for genotypes propagated in vivo and grown for 12 weeks on soil. **Data S9:**

Provides mean values and standard deviations for root and shoot weight measurements of different genotypes grown from seed for 12 weeks on soil. **Data S10:** Illustrates the influence of the propagation system (seed vs. in vivo) on genotype ranking. **Data S11:** Reports significance levels and *f* values for comparisons of regeneration performance, based on a phenotyping scale, among basal, middle and apical root explants cultivated in Jiffy swell pots. **Data S12:** Reports significance levels and *f* values for comparisons of regeneration performance, based on a phenotyping scale, among root explants at different time points during the experiment.