Cold stratification and type of rhizome affect the vegetative propagation and subsequent growth of *Falcaria vulgaris*

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Abstract

Falcaria vulgaris has not been domesticated or cultivated so far. The natural habitats of the medicinal plant F. vulgaris are endangered due to the use of herbicides and excessive harvesting by local people. Also, due to its therapeutic and food applications, there is a need for protocols for the propagation of *F. vulgaris*. To address this need, the experiment was performed as a two-factor factorial based on a completely randomized design with expe rimental factors being the type of rhizome (whole rhizome, proximal end of rhizome, and distal end of rhizome) and the period of cold stratification (one week, two weeks, and three weeks). The survival rate, number of days to emergence, fresh and dry weight, and the number of leaves, leaf length, leaf area, and crown diameter were measured as growth parameters. The best overall yield, in terms of survival rate, number of days to emergence, fresh and dry weight, number of leaves, leaf length, leaf area, and crown diameter, was observed in the proximal end of the rhizome (8 cm) and the whole rhizome propagules subjected to three weeks of cold stratification.

Keywords: *Falcaria vulgaris*, Proximal end, Rhizome sections, Whole rhizome, Natural habitant.

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Introduction

Falcaria vulgaris belongs to the order Apiales and the family Apiaceae (Taherzadeh et al., 2023). In the flora of Iran, the genus *Falcaria* includes two species: *F. vulgaris* Bornm. and *F. falcarioides* (Bornm. et Wolff) Wolff (Soleimani Shadvar and Moradkhani, 2022). Among these, *F. vulgaris* is primarily consumed as a vegetable. Due to the unique shape of its leaves, which resemble the webbed feet of a goose, it is called Paghaze and Ghaziaghi in Iran.

F. vulgaris is a perennial plant that grows as a rosette from late autumn to early spring, and during spring, its flowering stalk begins to grow from the middle of the rosette (bolting). The habitat of this plant is typically near agricultural fields (Monfared et al., 2012), and it is rarely found in areas far from cultivated lands.

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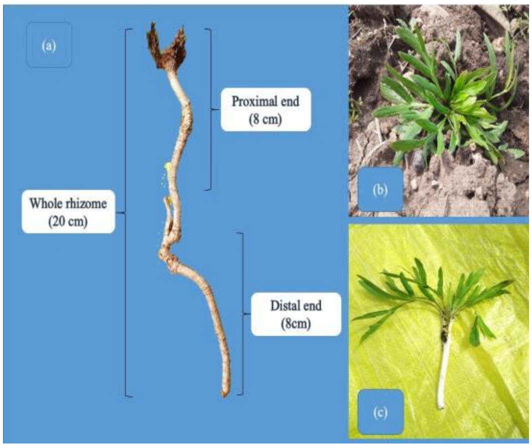

Fig. I. Different type of rhizome sections of Falacaria vulgaris used as propagules (a), Shoots emerging from rhizome (b), Crown of F. vulgaris as edible part of plants (c).

According to the literature, *Falcaria vulgaris* is distributed in temperate regions of Iran, including Lorestan, Kermanshah,Markazi, Hamedan, East Azerbaijan ,West Azerbaijan and Ardabil provinces (Hasanzadehfard et al., 2020). However, as far as we know, a comprehensive assessment of its natural habitats in Iran has not been conducted, and its distribution may be more extensive than currently documented.

In some areas of Iran, *F. vulgaris* is consumed as a vegetable during the middle of winter to early spring and is cooked with rice, eggs, or lentils. In the folk medicine of western Iran, this plant is used to heal skin wounds, treat stomach diseases including stomach ulcers, and address liver diseases as well as kidney and bladder stones (Khazaei et al., 2022). Additionally, *F. vulgaris* is recognized for its medicinal properties, including anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, and bleeding-inhibitory activities ((Sindhu et al., 2024). These medicinal effects require further evaluation in modern medicine.

According to Mapeka (2024), carvacrol (29.8%) is the main component of *F. vulgaris* essential oil, and its extract exhibits significant antimicrobial and antioxidant activity. In Iran, *F. vulgaris* is valued as a food source and is considered a good example of frugal food (Zangeneh et al., 2019). It has also been used occasionally as a flavoring for dairy products.

Despite its numerous food and medicinal applications, *F. vulgaris* has received little attention, and there has been no significant attempt to domesticate or cultivate it. Given the threats to its natural habitats, including overharvesting by local communities and herbicide use, this study aimed to develop a protocol for the propagation of this valuable plant.

Materials and Methods

Experimental Design

This two-factor factorial experiment consisted of a rhizome type factor (whole rhizome, proximal end of rhizome, and distal end of rhizome) and cold stratification at 4 °C (one week, two weeks, and three weeks) based on a completely randomized design (CRD). Each experimental treatment had three replicates, and the average of three plants was considered as one replicate. Data analysis was performed using the Statistical Analysis System program (SAS 9.4, SAS Institute Inc., NC, USA), and graphs were drawn using Excel 2013. Differences at a 5% probability level were considered significant.

Plant Material

Rhizomes of *Falcaria vulgaris* were collected from the barley and wheat fields near Kouhdasht city, Lorestan province, Iran, in early autumn. The rhizomes were washed with distilled water for the experiment. Complete rhizomes, the proximal end (8 cm), and the distal end (8 cm) were cut and used as propagules (Fig. I a). After cutting the rhizomes, they were treated with benomyl fungicide (at 100 g/100 L) for 1 h, followed by three rinses with distilled water. Gardening sand with a particle diameter of 2 to 5 mm was used as a cold stratification medium. After disinfection, the cuttings were placed in moist gardening sand and kept at 4 °C.

Culture Medium

The soil was plowed to a depth of 45 cm for the cultivation of rhizomes. To determine the soil properties, samples were collected and transferred to the laboratory for analysis. The results of the soil test are presented in Table 1. The rhizomes were cultured horizontally at a depth of

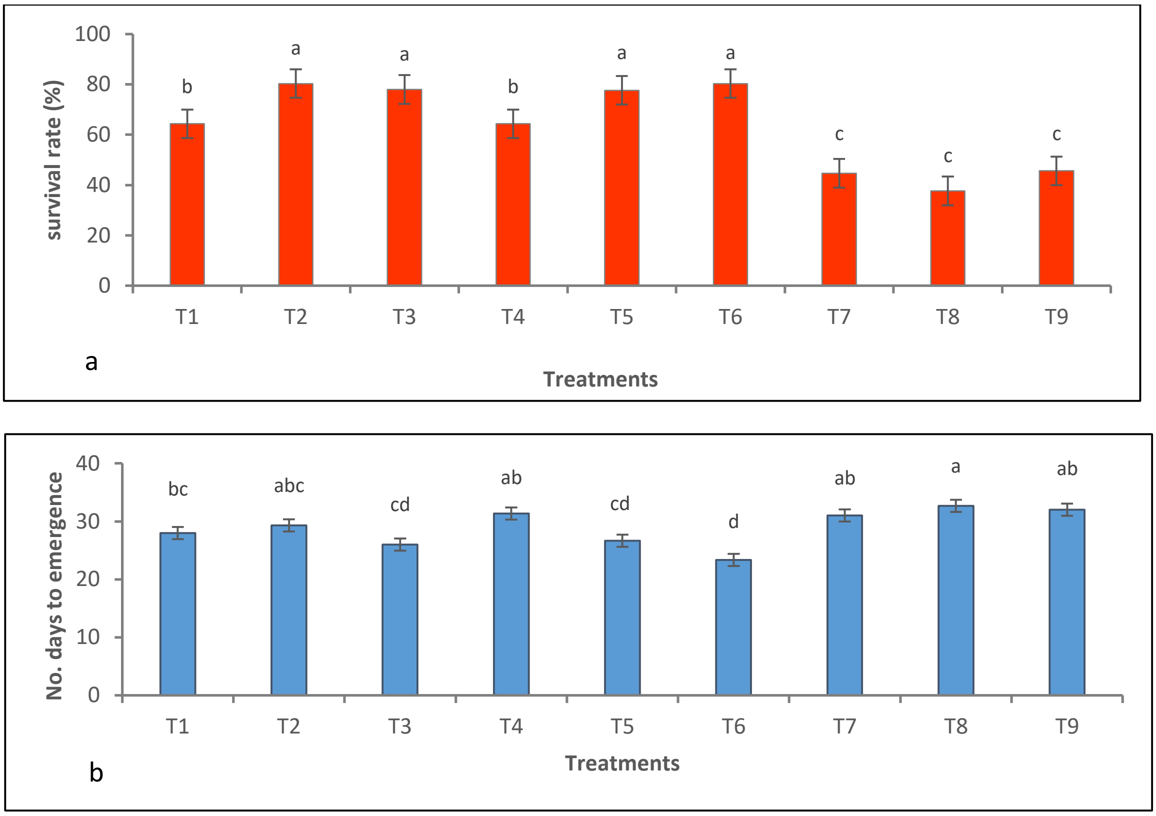

Fig. II. Effect of rhizome type and cold stratification period (4 °C) on the survival rate of propagules (a) and no. days to plant emergence (b): (T1) W+ one-week cold; (T2) W+ two-week cold; (T3) W+ three-week cold; (T4) P+ one week cold; (T5) P+ two-week cold; (T6) P+ three-week cold; (T7) D+ one week cold; (T8) D+ two-week cold; (T9) D+ three-week cold. The same letters designate values with differences not reliable at $P \leq 0.05$. W: whole rhizome, P: proximal end of rhizome, D: distal end of rhizome.

7 cm on ridges, spaced approximately 20 cm apart with 10–15 cm between plants, and then irrigated. After rhizome cultivation, a low tunnel (60 cm high) was used. The tunnel was covered with a polyethylene film with a light transmission rate of 87%. Since *F. vulgaris* is a temperate plant capable of withstanding sub-zero temperatures, no heating system was used.

Growth Analysis

After about a month, most of the rhizomes produced leaves. The number of rhizomes that produced crowns was recorded as the survival rate of rhizomes. The number of days until the emergence of the first sign of rhizome growth was also noted. Forty-five days after rhizome cultivation, additional growth-related analyses were performed. The fresh weight of each crown was recorded as the fresh weight. Each crown was dried at 65 °C for 48 h until a constant weight was achieved, and the dry weight (DW) was determined. A destructive method was used to measure leaf area (LA) values of different treatments using a Leaf Area Meter (DELTA–T, UK). The number of leaves was counted, and the average length of fully expanded leaves was recorded.

Results

In this experiment, growth occurred under all treatments. The survival rate of rhizomes was evaluated and analyzed. The highest survival was achieved when the proximal end of the rhizomes and the whole rhizome were subjected to extended periods of cold stratification (T2, T3, T5, and T6). The lowest survival rate was obtained when the distal end of the rhizomes was used, regardless of the cold stratification period (T7, T8, and T9) (Fig. II a). The shortest time from rhizome cultivation to plant emergence was observed in the combination of the proximal end with three

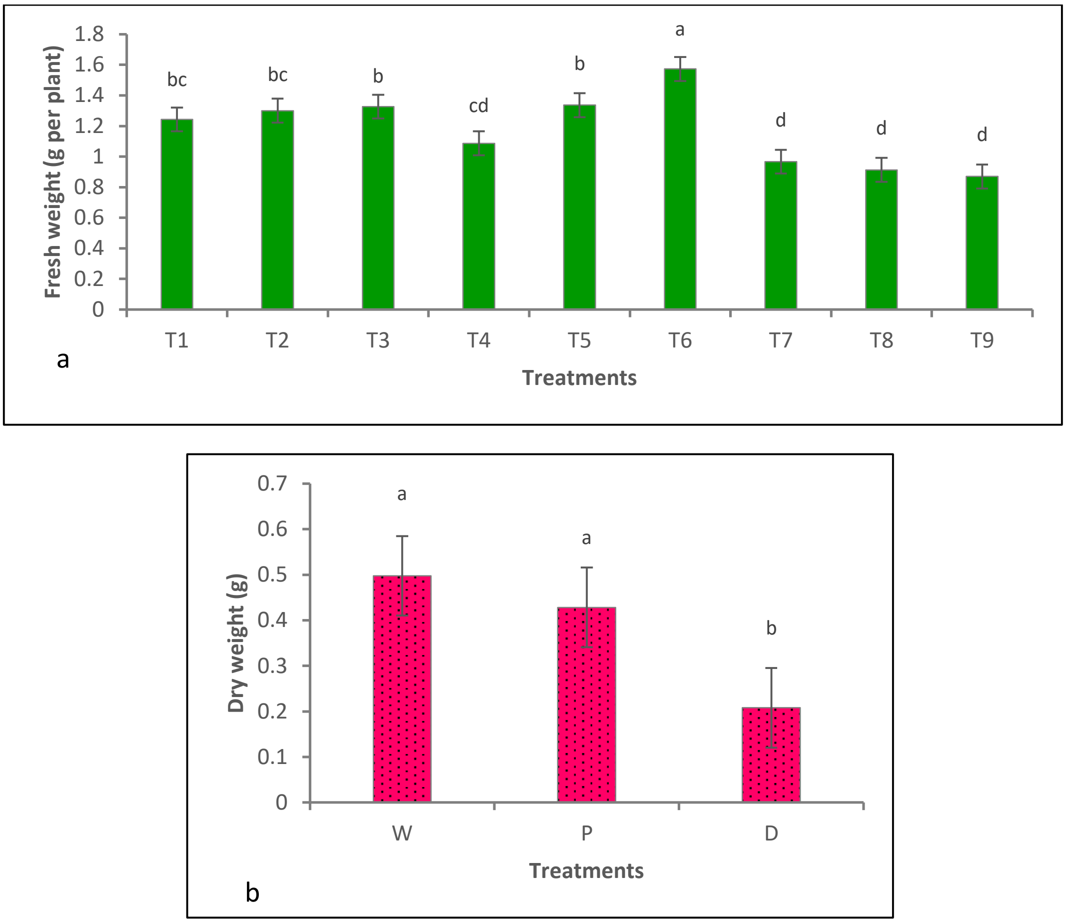

Fig. III. Effect of rhizome type and cold stratification period (4 °C) on the fresh (a) and dry weight (b) of plant edible parts: (T1) W+ one-week cold; (T2) W+ two-week cold; (T3) W+ three-week cold; (T4) P+ one week cold; (T5) P+ two-week cold; (T6) P+ three-week cold; (T7) D+ one week cold; (T8) D+ two-week cold; (T9) D+ three-week cold. The same letters designate values with differences not reliable at P ≤ 0.05. W: whole rhizome, P: proximal end of rhizome, D: distal end of rhizome.

weeks of cold stratification (T6). The longest time to plant emergence was related to the distal end of the rhizome with two weeks of cold stratification (T8) (Fig. II b).

According to the results of this experiment, it is recommended that the proximal end of the rhizome and the whole rhizome be cultured after three weeks of cold stratification (4 °C). The interaction effect of both treatments on the fresh weight was also significant. The highest fresh weight of the crown (edible parts) was obtained from the proximal end of rhizomes under three weeks of cold stratification (T6). The lowest fresh weight was obtained when the distal end of the rhizomes was used as the propagule (T7, T8, and T9) (Fig. III a).

Analysis of the results showed that the interaction of both factors on dry weight was not significant, and this trait was mainly dependent on the type of rhizome propagules. The highest dry weight was obtained when the whole rhizome was used as the propagule, and the lowest dry weight was obtained when the propagule was the distal end of the rhizomes (Fig. III b).

The results of the analysis showed that the interaction of both factors had a significant effect on the number of leaves. More leaves were achieved when the whole rhizome was cultured as propagule material. Three weeks of cold stratification also seemed to increase the number of leaves (T1 and T3). The lowest number of leaves was observed when the distal end of the rhizomes underwent one week of cold stratification (T7) (Fig. IV a).

Leaf length was not affected by cold stratification or the interaction of both factors. The highest leaf length was observed when the whole rhizomes were used as propagule material (Fig. IV b). The

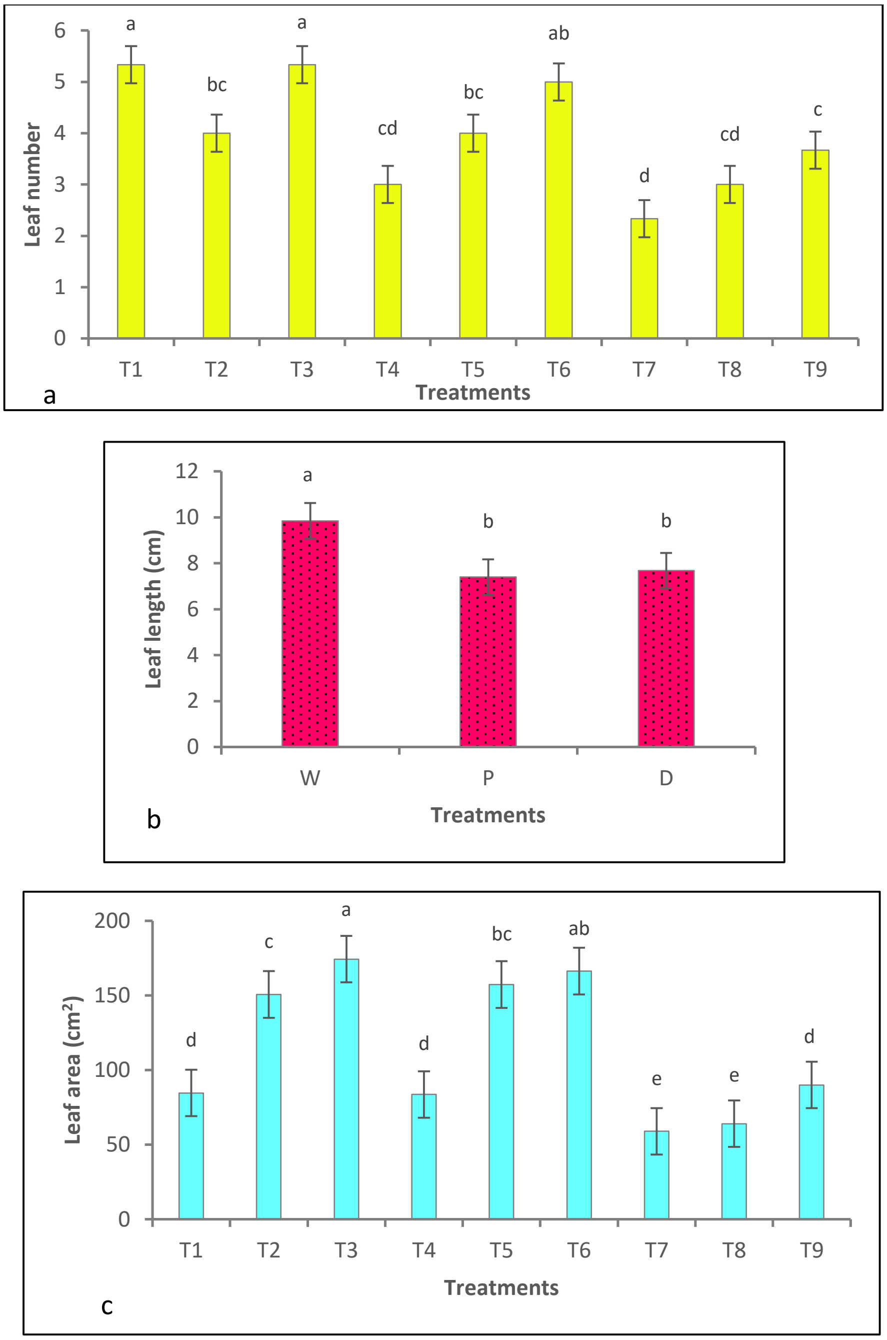

Fig. IV. Effect of rhizome type and cold stratification period (4 °C) on the leaf number (a), leaf length (b), and leaf area (C) of plant: (T1) W+ one-week cold; (T2) W+ two-week cold; (T3) W+ three-week cold; (T4) P+ one week cold; (T5) P+ two-week cold; (T6) P+ three-week cold; (T7) D+ one week cold; (T8) D+ two-week cold; (T9) D+ three-week cold. The same letters designate values with differences not reliable at $P \leq 0.05$. W: whole rhizome, P: proximal end of rhizome, D: distal end of rhizome.

leaf area (LA) was significantly affected by the interaction of both factors. The lowest leaf area (LA) was obtained when the distal ends of the rhizomes were used as propagules, with cold stratification of one and two weeks, respectively (T7 and T8). The highest leaf area (LA) was observed in whole rhizomes under three weeks of cold stratification (T3 and T6) (Fig. IV c).

The crown diameter of this plant was not affected by the interaction of both factors. However, the main effects of both factors significantly affected this trait. The crown diameter was highest when

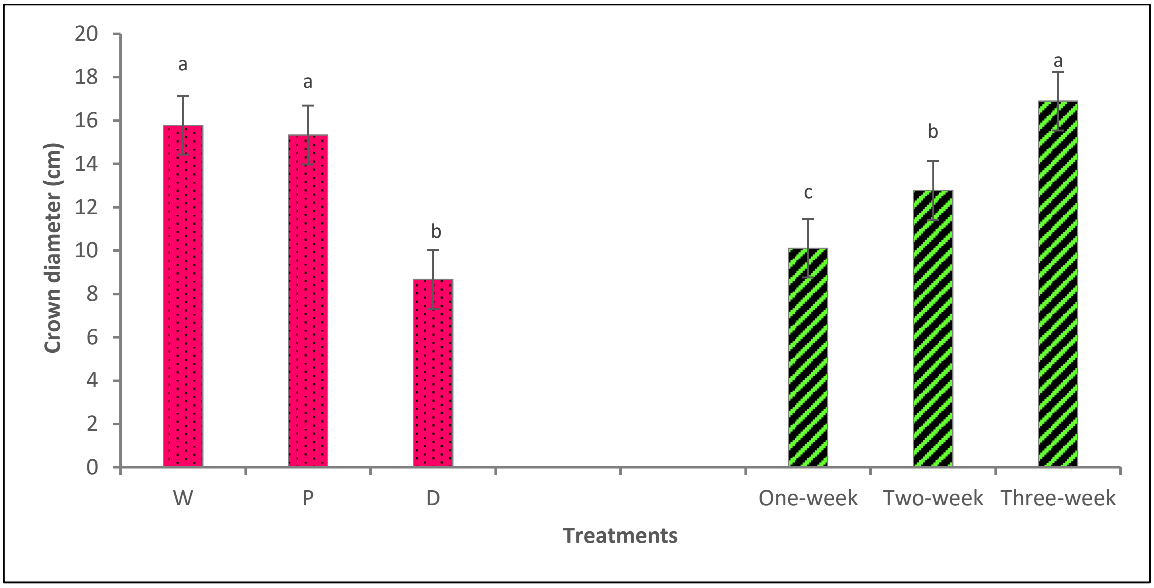

Fig. V. Effect of rhizome type and cold stratification period (4 °C) on plant crown diameter: (T1) W+ one week cold; (T2) W+ two-week cold; (T3) W+ three-week cold; (T4) P+ one week cold; (T5) P+ two-week cold; (T6) P+ three-week cold; (T7) D+ one-week cold; (T8) D+ two-week cold; (T9) D+ three-week cold. The same letters designate values with differences not reliable at P ≤ 0.05. W: whole rhizome, P: proximal end of rhizome, D: distal end of rhizome.

the proximal end of the rhizomes and the whole rhizomes were used as propagule material (Fig. V). With increasing duration of cold stratification, the crown diameter also showed an upward trend (Fig. V).

Based on these results, it can be stated that the distal end of the rhizome is not a suitable propagule for the propagation of *Falcaria vulgaris*, and it is better to use whole rhizomes or 8 cm proximal ends of the rhizomes for the propagation of *F. vulgaris*.

Discussion

The lowest time to seedling emergence was associated with T6, T5, and T3, respectively. However, the longest emergence time was associated with the distal end of the rhizome. The results of our experiment showed that a whole rhizome improves the rate of emergence. These findings suggest that the proximal end of the rhizome and the whole rhizome may contain more developed buds for faster growth as propagules, and cutting the proximal end of the rhizome may help to quench rhizome dormancy(Nin et al., 2021). Mangoale and Afolayan (2020) reported that as the length of the rhizome propagule of the medicinal plant *Alepidea amatymbica* increased, the emergence rate also increased. One justification for this finding is that long rhizomes have a higher carbohydrate content (Zhu et al., 2022)and that subsequent survival and growth may depend on these carbohydrate pools ((Li et al., 2021).

Our results showed that a longer duration of cold stratification causes the plant to emerge faster. Various reports have shown that cold stratification converts insoluble sugars into soluble sugars such as sucrose (Wu et al., 2024). These soluble sugars provide metabolic energy early in the growing season (Afzal et al., 2021).

The highest survival rate occurred when the whole rhizome and the proximal end of the rhizome, under a longer cold stratification period, were used as propagules. Pereira et al.(2024)achieved better results in *Alstroemeria* sp. micropropagation under in vitro conditions when they used rhizome tips as explants. One reason could be attributed to greater activity at the apex and meristemic points of the rhizomes. However, there are limited studies on the effect of rhizome origin on plant propagation. (Kumar et al.(2024)found that the apical part of the rhizomes of *Trillium govanianum* had the ability to develop roots and buds, while other parts of the rhizome did not survive.

Fresh weight in T6 was higher than in all other treatments. This may be due to early emergence, as in this treatment, the leaves appeared earlier on the soil surface. Dry weight was slightly higher when the whole rhizome was used as propagule material, which was not significantly different from the proximal end of the rhizome. One justification for this finding is that dry weight gain may be due to the high nutritional and carbohydrate content of whole rhizomes. Our results are consistent with those of Mangoale and Afolayan (2020), who reported that *Alepidea amatymbica* growth was greater when larger rhizomes were planted.

The interaction of cold stratification and propagule type significantly affected leaf number and leaf area. T1, T2, and T6 treatments had the highest number of leaves. The early emergence of seedlings may have increased the number of leaves. When the rhizome was used as a whole propagule, the leaf length was longer than in other propagules. Leaf area was greatest in T3 and T6, respectively. Leaf development is very important, as leaves represent the primary edible part of the *F. vulgaris* plant. Our results are consistent with Makena et al.'s findings on *Helichrysum odoratissimum* (Makena et al., 2023).

The crown diameter of the *F. vulgaris* rosette was also affected by the type of propagule and the cold treatment, but not by their interaction. The proximal end of the rhizome and the whole rhizome had the largest crown diameter and showed an increase in crown diameter with increasing cold stratification duration. Our results are similar to those of Chukwudi et al.(2020), who observed greater biomass production when large-sized propagules were grown in *Zingiber officinale* L. The number of leaves produced in the longer rhizome may result from rhizome length being positively correlated to levels of carbohydrates, proteins, and other nutrients (Lopez et al., 2023). Cold stratification also dissolves insoluble carbohydrates (Wu et al., 2024), thus providing materials needed for metabolism to bursting buds.

The ability of the rhizome apex to propagate *Trillium govanianum*, an endangered medicinal plant, has also been recently reported by Chauhan et al. (2020), which aligns with our results.

Conclusion

For the first time, we tried to develop a low-cost protocol for the propagation of *F. vulgaris*. The results of the study indicated that the type of propagule and the duration of cold stratification significantly affect the survival and subsequent growth of *F. vulgaris* plantlets. Overall, it is recommended to use an 8 cm section of the proximal end of the rhizome or the whole rhizome under three weeks of cold stratification at 4 °C for the vegetative propagation of this plant. These results could play a crucial role in establishing propagation protocols for *F. vulgaris*. Vegetative propagation using rhizomes may help meet current and future demands for *F. vulgaris*.

Author Contributions

YF carried out the experiments, collected the data, and wrote the paper. DR conceptualized the study and presented the main idea. AAA helped in data analysis and presentation. All the authors read and approved the final version of the manuscript.

Compliance with Ethical Standards Conflict of Interest

The authors declare that they have no conflict of interest.

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