

## ***Ferula gummosa*: Compatibility of Cultivating at Different Planting Dates**

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### **ABSTRACT**

A factorial experiment, employing a completely randomized design with three replications, was conducted during the 2018-2019 growing season at the laboratory and greenhouse of Islamic Azad University, Shirvan Branch. The study aimed to evaluate the feasibility of cultivating the medicinal plant Galbanum (*Ferula gummosa* Boiss) at various planting dates. The first factor was the planting date, which included four levels: February 20, March 6, March 21, and April 4. The second factor involved four seed treatments: control, primed seeds, dormant seeds, and tissue culture. The traits measured in the study were leaf number, leaf length, tuber length, dry weight, and survival percentage. Results indicated that both planting date and seed treatment significantly affected all measured traits ( $P \leq 0.01$ ). Additionally, the interaction between planting date and seed treatment significantly influenced survival percentage ( $P \leq 0.05$ ). Mean comparisons showed that the highest values for leaf number, leaf length, tuber length, dry weight, and survival percentage were achieved with the February 20 planting date combined with the seed priming treatment. Correlation analysis revealed significant positive relationships between leaf length and tuber length (0.879), leaf length and plant dry matter content (0.522), tuber length and plant dry matter content (0.707), tuber length and survival percentage (0.406), and plant dry matter content and survival percentage (0.509). Stepwise regression analysis indicated that plant dry matter content was the first variable included in the model, accounting for 26% of the variation in survival percentage. Leaf number was the next variable, and together, these two traits explained 33% of the variation. Even these findings, and considering the importance of planting date for cultivating Galbanum in Northern Khorasan Province, the February 20 planting date combined with seed priming is recommended as the optimal treatment for maximizing production.

**Keywords:** Galbanum, Planting Date, Cultivation, Priming, Path analysis

### **INTRODUCTION**

Medicinal plants are among Iran's most valuable natural resources. With appropriate scientific recognition, cultivation, development, and utilization, they can significantly contribute to public health, job creation, and non-oil exports [1, 2]. Understanding the benefits and costs of various cropping systems for medicinal plants is essential for making informed decisions about species conservation, whether in natural habitats, on farms, or both [3, 4]. Cultivation and wild collection should be seen as complementary strategies rather than mutually exclusive options, requiring thorough investigation [5]. A thorough understanding of the growth stages and factors influencing the production of medicinal plants is fundamental for their sustainable production and use [6].

The planting date is a crucial management practice that greatly impacts yield and other agronomic traits. The goal of determining the optimal planting dates is to synchronize sowing with favorable environmental conditions that promote seed germination, seedling establishment, and plant survival [7]. In Khorasan Province, 37 Galbanum habitats have been identified, spanning approximately 171,500 hectares. These habitats produce an estimated 100 tons of Galbanum resin each year, resulting in a gross income of around one billion Rials [8].

Galbanum (*Ferula gummosa* Boiss) is a perennial, monocarpic plant from the Apiaceae family. This valuable medicinal plant is used in a range of products, including pharmaceuticals, insecticides, perfumes, and industrial applications, such as the production of smooth, transparent glue for the jewelry industry [9, 10]. Traditionally, it is used to treat stomach worms and enhance milk production. As a native plant of Iran, its resin is an important export commodity with various medical applications [11].

Breaking seed dormancy in *Ferula gummosa* requires a stratification process that involves a cold period of 15 to 45 days at temperatures between 3 and -15 degrees Celsius [12]. Chilling treatment is sometimes applied alone or in combination with other treatments, such as gibberellic acid, to break dormancy and enhance germination [13]. Nitrogen-containing compounds, including potassium nitrate, are commonly used in seed testing laboratories to treat dormant seeds [14].

Rapid and uniform seed germination, along with seedling emergence, is essential for successful establishment. Seed priming is a valuable technique that enhances both the speed and uniformity of germination while also improving seed tolerance in challenging environmental conditions [15, 16]. Given that traditional propagation methods are often insufficient to meet current demands, it is necessary to adopt newer methods as replacements or complements [17]. Plant biotechnology provides innovative methods such as tissue culture and grafting, allowing for the rapid and large-scale multiplication of plants while safeguarding valuable genetic resources [18, 19]. These techniques can also facilitate the production of uniform plants, increase secondary metabolite production, and aid in conserving endangered species or valuable genotypes [20, 21].

Galbanum is economically important due to its contributions to exports and the livelihoods it supports. However, populations of this wild medicinal plant are declining in many rangelands because of uncontrolled harvesting, leading to irreparable damage to Iran's valuable

national resources. This study explores the potential for domesticating and cultivating Galbanum (*Ferula gummosa* Boiss) as a systematic conservation strategy. To achieve this, seeds of *Ferula gummosa* from the Northern Khorasan region were planted in pots at various times to assess their response and identify suitable cultivation areas.

## **MATERIALS AND METHODS**

### **Plant materials and Treatments**

A factorial experiment utilizing a completely randomized design with three replications was conducted to explore the feasibility of cultivating Galbanum under greenhouse conditions. Seeds of *Ferula gummosa* were sourced from the North Khorasan Agricultural and Natural Resources Research Center. The primary objectives of this thesis were to assess tissue culture, grafting, dormancy breaking, seed priming, and the domestication potential of the Galbanum medicinal plant (*Ferula gummosa* Boiss) from the North Khorasan massif. This research took place in 2017 and 2018 at the laboratory of Azad University in Shirvan. To break dormancy and prime the Galbanum seeds, the seeds were initially stratified for three weeks at 4°C in the university's laboratory refrigerator. For the domestication phase, a bud from a tissue culture experiment, plants from dormancy-breaking and seed priming experiments, and second-year plants obtained from transplants were utilized. The experiment involved two main factors: the planting date, which had four levels (February 20, March 6, March 21, and April 4), and four seed treatment types (control, tissue culture, dormancy-broken seeds, and primed seeds).

### **Control Treatment**

The control treatment involved untreated *Ferula gummosa* seeds that were planted directly, without any pre-sowing manipulation. These seeds were sourced from the North Khorasan Agricultural and Natural Resources Research Center and stored under standard laboratory conditions until planting. In the control group, three untreated seeds were sown directly into each plastic pot at a depth of 1 cm. The potting mixture comprised 50% field soil, 25% leaf litter, and 25% sand. No chemical or physical treatments were applied to the seeds prior to planting. This control treatment established a baseline for comparing the effectiveness of other seed treatments on germination, growth parameters, and survival percentage throughout the experiment.

### **Primed Seeds Treatment**

Seed priming was conducted to improve the germination rate and uniformity of *Ferula gummosa*. The seeds underwent hydro-priming with a zinc sulfate solution to enhance metabolic activity prior to planting. This process involved soaking the seeds in the solution for a specific duration at controlled temperatures, followed by surface drying to restore their original moisture content. The goal was to activate pre-germination metabolic processes without causing radicle protrusion. Primed seeds exhibit faster and more synchronized emergence, increased seedling vigor, and enhanced tolerance to environmental stresses during establishment. After priming, three treated seeds were planted per pot at a depth of 1 cm in the same potting mixture used for the control treatment.

### **Dormancy-Broken Seeds Treatment**

Breaking seed dormancy in *Ferula gummosa* necessitates cold stratification due to the species' physiological dormancy mechanism. Seeds obtained from the North Khorasan Research Center underwent moist stratification at 4°C for three weeks in the university laboratory refrigerator. This cold treatment simulates the natural winter conditions that the seeds would encounter in their native habitat, which is crucial for initiating germination. During stratification, embryo development occurs, and hormonal changes take place, specifically a decrease in abscisic acid levels and an increase in gibberellins. After the three-week cold period, the dormancy-broken seeds were immediately planted in pots at a depth of 1 cm to assess their germination response and subsequent growth performance.

### **Tissue Culture Treatment**

The tissue culture treatment utilized micropropagation techniques to generate genetically uniform *Ferula gummosa* plantlets under sterile conditions. Seeds were sourced from mature Galbanum plants at the North Khorasan Research Center and were cultured on nutrient media enriched with growth regulators, including auxins and cytokinins. The cultures were maintained in controlled environmental conditions with specific light and temperature settings to promote shoot proliferation and root development. Once successfully established in vitro, the plantlets underwent a gradual acclimatization process to adapt to ex vitro conditions. The hardened plantlets were then transferred to greenhouse pots filled with a standard potting mixture. This biotechnological approach allows for rapid multiplication while preserving valuable genetic resources and ensuring species uniformity.

Plants from all experiments were moved to the greenhouse. For the greenhouse experiments, plastic pots with a capacity of approximately 1 kg were filled with a potting mixture made up of 50% field soil, 25% leaf litter, and 25% sand [22].

In each pot, three germinated seeds with healthy radicles were planted 1 cm deep. At the same time, three untreated seeds were directly planted in the control pots. The pots were examined every three days, and the number of emerged plants in each pot was recorded. Observations for assessing seed germination and seedling establishment were made over a 35-day period [23]. At the end of the growing season, when the aerial parts of the plants had dried, the storage roots—produced during that season—were harvested from each pot and transferred to the laboratory. There, the survival percentage was recorded (Fig. 1), the number of leaves was counted, and the length of the storage roots was measured. The aerial and underground parts were then placed in separate bags and dried at 75°C for 72 hours [18].



**Fig. 1** The stages of Galbanum domestication experiment

### Statistical Analysis

The experiment utilized a completely randomized design with three replications. Before conducting the analysis of variance, the PROC UNIVARIATE procedure assessed the normality of the data distribution. The statistical analysis began with descriptive statistics to evaluate the quality of the collected data and simplify the extensive datasets for easier interpretation. More complex analyses followed, including the computation of mean squares and experimental errors. Duncan's Multiple Range Test (DMRT) at a significance level of  $P \leq 0.05$  was employed to compare means using SPSS software (v. 26; IBM Corp., Armonk, NY, USA). Pearson's correlation coefficient ( $r$ ) was calculated to assess correlations between different trait pairs with the same software. Additionally, stepwise regression analysis at  $P < 0.01$  was conducted to identify key traits influencing Galbanum survival percentage. Finally, path analysis was performed to examine the direct and indirect effects of each trait retained in the regression model. All statistical analyses were conducted using SPSS V.26 software.

### Results and Discussion

#### Descriptive Statistics Analysis

Descriptive statistics, including mean, standard error (SE), minimum, maximum, range, and phenotypic coefficient of variation (PCV), are summarized in Table 1. The dimensionless nature of PCV allows for easier comparison among traits. The PCV values were 6.34% for leaf number, 4.12% for leaf length, 3.13% for plant dry matter content, 2.89% for tuber length, and 2.74% for survival percentage. Table 1 indicates that survival percentage had the highest range; however, it did not have the highest PCV. This highlights the significance of using dimensionless indices, such as CV, for comparing trait variability. A high PCV signifies substantial variation among treatments for a given trait. Overall, the results of this experiment demonstrated considerable variation among treatments for leaf number, leaf length, plant dry matter content, tuber length, and survival percentage.

**Table 1** Descriptive statistics related to the various traits in Galbanum.

Trait	Mean $\pm$ SE	PCV (%)	Minimum	Maximum	Range
Leaf number	3.95 $\pm$ 0.09	6.34	3	5	2
Leaf length	11.7 $\pm$ 0.41	4.12	6	17	11
Tuber length	9.31 $\pm$ 0.46	2.89	3	15	12
Plant dry matter content	5.29 $\pm$ 0.24	3.13	2.41	8.8	6.39
Percentage survival	29.36 $\pm$ 1.56	2.74	11	55	44

#### Comparison of Means Analysis

##### Leaf Number

ANOVA results demonstrated a significant effect of planting dates on leaf number ( $P < 0.01$ , Table 2). The highest leaf number was recorded for the February 20 planting date (4.41), while the lowest was for the April 4 planting date (3.5) (Fig 2, Table 3). Additionally, cultivation practices significantly influenced the leaf number per plant ( $P \leq 0.01$ , Table 2). Plants derived from tissue culture had the highest leaf number (5.33), whereas the control treatment showed the lowest (3.25) (Table 4). Earlier planting dates resulted in longer growth and development, ultimately leading to an increased number of leaves per plant [24, 25]. Conversely, delayed planting resulted in a shorter vegetative period and fewer leaves, suggesting that variations in leaf number were due to the duration of vegetative growth [26].

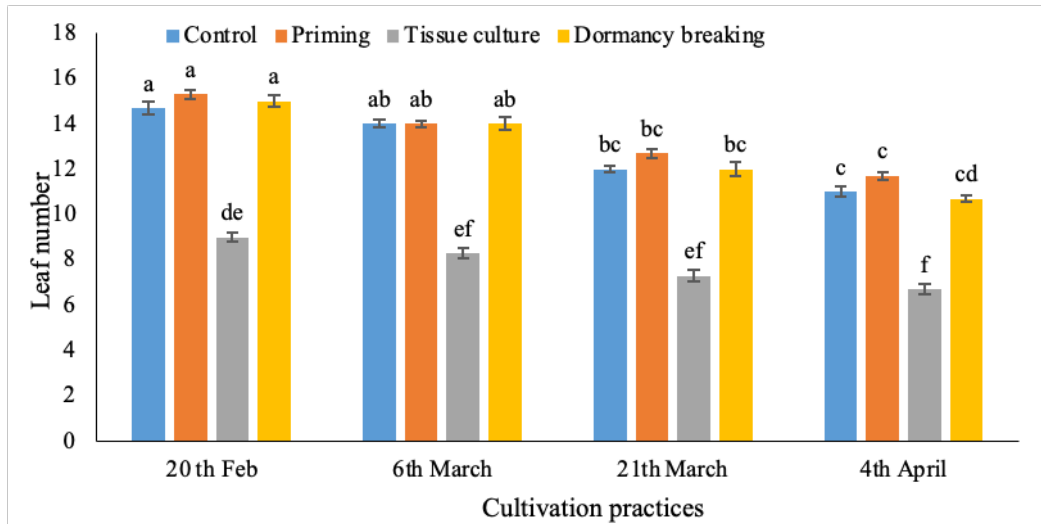


Fig. 2 Interaction effect of planting date and cultivation practices on leaf number

Table 2 ANOVA results of the studied traits of Galbanum

Sources of variation	Degree of freedom	Leaf number	Leaf length	Tuber length	Plant dry weight	Survival percentage
Planting date (a)	3	1.74 *	29.52 **	33.96 **	3.22 *	221.07 **
Cultivation practices (b)	3	10.46 **	83.35 **	118.96 **	29.01**	926.35 **
a x b	9	0.40 **	0.50 **	1.78 **	0.78 *	85.96**
Error	32	0.27	1.16	0.45	0.90	35.22
CV (%)	-	13.07	9.17	7.20	18.05	20.33

ns, \* and \*\* represent not significant and significant at the 5 and 1% probability, respectively.

Table 3 Comparison of the mean effects of the various treatments on the studied traits

Treatment/trait	Leaf number	Leaf length (cm)	Tuber length (cm)	Plant dry matter content (g)	Percentage survival
Feb. 4, 2018	4.41 a	13.5 a	11.5 a	5.96 a	33.75 a
Mar. 6, 2018	3.91 b c	12.58 b	9.91 b	5.31 ab	31.97 a
March 21, 2018	4.08 ab	11 c	8.5 c	5.07 b	26 b
Apr. 4, 2018	3.5 c	10 d	7.66 d	4.74 b	25.08 b
Control	3.25 c	12.91 a	9 c	3.9 b	17.75 c
Priming	3.83 a	13.41 a	12.41 a	6.72 a	16.39 a
Tissue culture	5.33 a	7 b	5.16 d	3.95 b	66.29 b
Dormancy breaking	3.5 b c	12.91 a	11 b	6.5 a	16.30 b

The means having similar letters in each column are not significantly different in Duncan's test at the 5% level.

### Leaf Length

ANOVA indicated that planting dates significantly influenced leaf length ( $P < 0.01$ , Table 2). The longest leaves measured 13.5 cm and were produced on February 20, while the shortest leaves, at 10 cm, were observed on April 4. Additionally, cultivation practices had a significant impact on leaf length ( $P \leq 0.01$ , Table 2). The priming treatment resulted in the longest leaves at 13.41 cm, whereas tissue culture-derived plants had the shortest leaves, measuring 7.83 cm (Table 4, Fig 3). Early planting likely facilitated more complete canopy cover, leading to greater light interception due to the larger leaf area and, as a result, increased leaf length [6, 27, 28]. The results suggest that this plant requires temperatures below 30°C for optimal growth, performing best in May when temperatures are moderate and rainfall is substantial, which aligns with field observations and simulations [29].

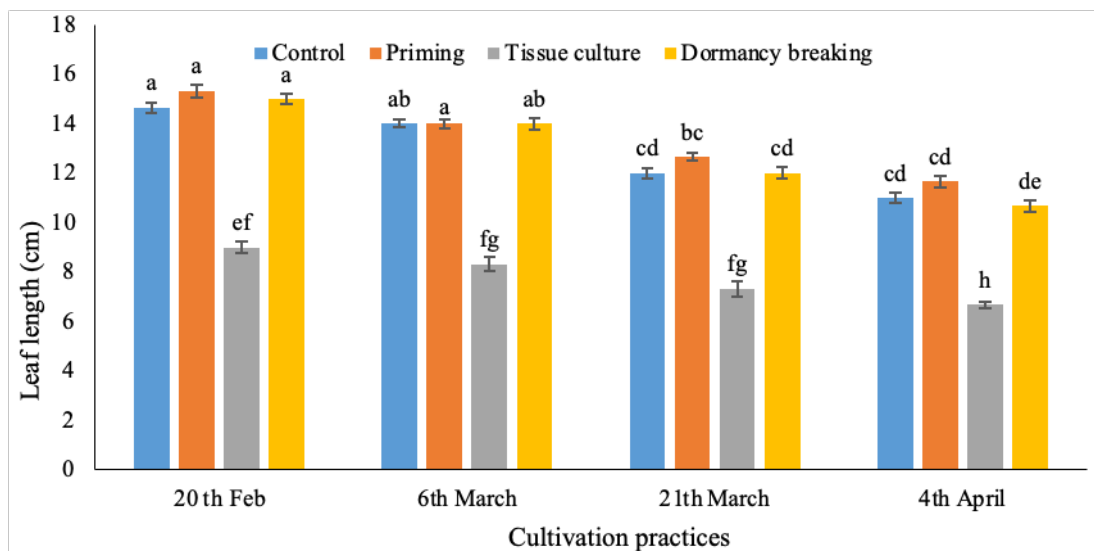


Fig. 3 Interaction effect of planting date and cultivation practices on Leaf length

### Tuber Length

The planting date had a significant impact on tuber length ( $P < 0.01$ , Table 2). The longest tubers were observed from the February 20 planting, while the shortest measured 7.66 cm and were from the April 4 planting (Table 3). Additionally, the cultivation method also significantly influenced tuber length ( $P \leq 0.01$ , Table 2). The priming treatment produced the longest tubers at 12.41 cm, whereas tissue culture resulted in the shortest at 5.16 cm (Table 4). The timing of planting greatly affects plant growth and development due to variations in day length, temperature, and humidity, making it a crucial management practice. The February 20 planting date yielded the highest germination rate and optimal plant growth, indicating its suitability for primed seeds in Northern Khorasan. This timing facilitates better establishment and dry matter accumulation (Fig 4). Conversely, delaying planting is likely to decrease the survival percentage [30-32].

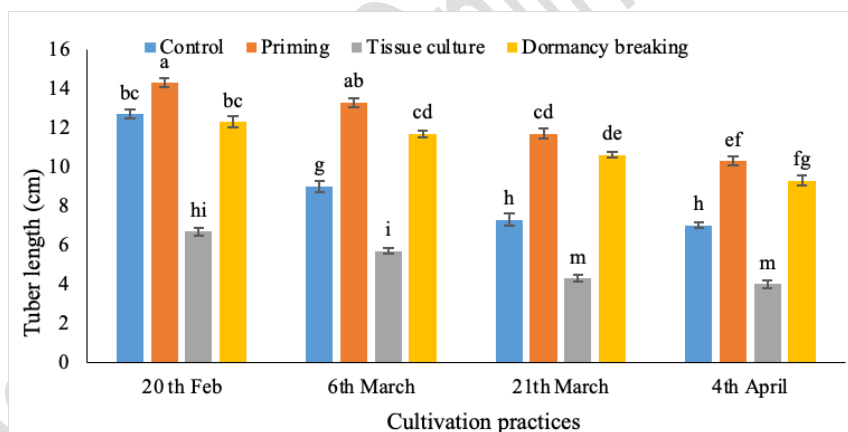


Fig. 4 Interaction effect of planting date and cultivation practices on Tuber length

### Plant Dry Matter

The planting date had a significant impact on plant dry matter content ( $P < 0.05$ , Table 2). The highest dry matter recorded was 5.96 g from the February 20 planting date, while the lowest was 4.74 g from the April 4 planting date. Additionally, cultivation practices significantly influenced dry matter content ( $P \leq 0.01$ , Table 2). The priming treatment yielded the highest dry matter at 6.72 g, compared to the control, which produced the lowest at 3.9 g (Table 4, Fig 5). These results are consistent with previous findings that priming beans with zinc sulfate enhances growth and increases dry matter [33]. Early planting extends the growing season, allowing plants to benefit from favorable conditions, expand leaves faster, intercept more sunlight, and increase dry matter production [34].

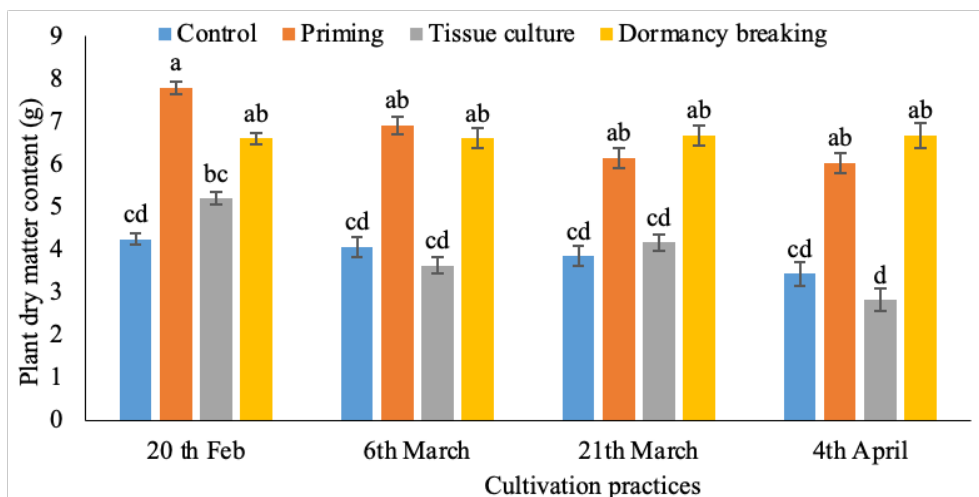


Fig. 5 Interaction effect of planting date and cultivation practices on Plant dry matter

### Survival Percentage

The interaction between planting date and cultivation practice significantly affected the survival percentage ( $P \leq 0.05$ , Table 2). The highest survival rate of 48.66% occurred with the combination of planting on February 20 and the priming treatment, while the lowest rate of 15.33% was observed for the April 4 planting with the control treatment (Fig. 6). Early planting provides ample time for growth, leaf senescence, and tuber reserve accumulation, which enhances cold tolerance and promotes vigorous regrowth in the following year [6]. Delayed planting reduces the vigor of aerial parts and decreases survival [35]. Primed seeds consistently performed better, accumulating more reserves by season's end [36]. This superiority may stem from improved branching and canopy development, which could be associated with the role of nutrients such as potassium in directing non-structural carbohydrates to the roots and stems [21, 37, 38]. Larger storage organs with greater tuber weight better guarantee regrowth [39], a finding consistent with research on other root and tuber crops [39-41].

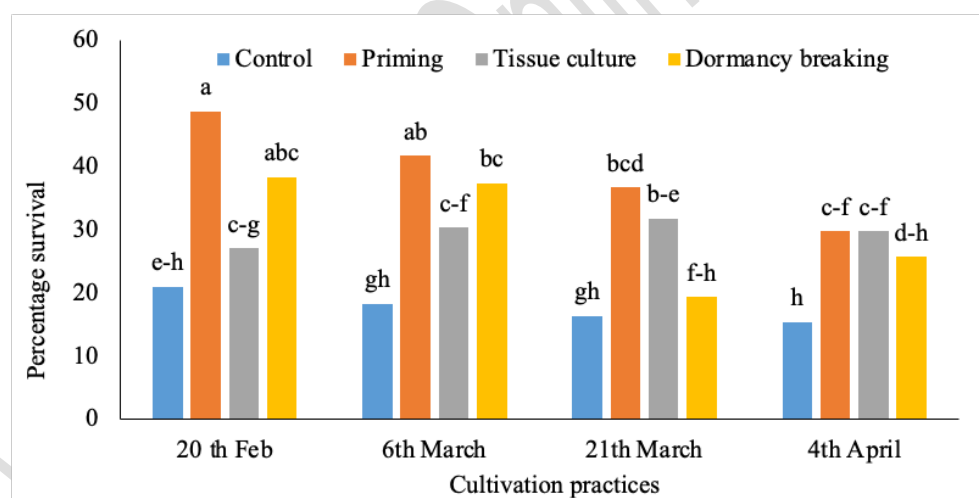


Fig. 6 Interaction effect of planting date and cultivation practices on survival percentage

### Correlation Coefficients Analysis

Pearson's correlation coefficients among growth attributes are presented in Figure 7. Significant positive correlations were found between leaf length and tuber length (0.879), leaf length and plant dry matter content (0.522\*), tuber length and plant dry matter content (0.707), tuber length and survival percentage (0.406), and plant dry matter content and survival percentage (0.509).

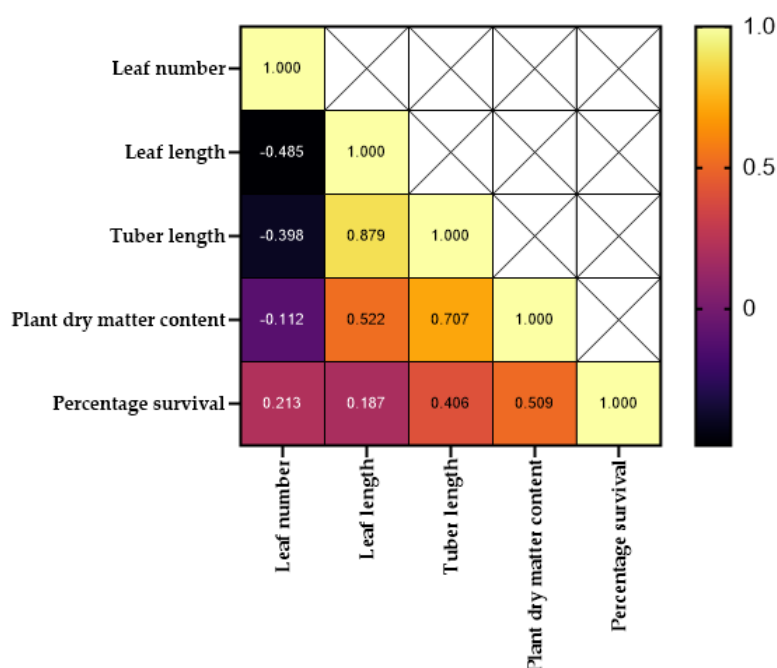


Fig. 7 Heat map of mutual relations of variables in correlation coefficient for triats indicators.

The results showed that leaf length with tuber length (0.879\*\*), plant dry matter content (0.522\*); tuber length with plant dry matter content (0.707\*\*) and percentage survival (0.406\*); plant dry matter content with percentage survival (0.509\*) had the highest positive and significant correlation. The coefficient of variation and linear regression are essential tools for analyzing the relationship between linear changes in two variables, particularly in plant breeding studies. Research has shown a significant positive correlation between ion leakage and water core in citrus cultivars. Understanding these correlations can assist plant breeding researchers in indirectly selecting important stress traits by focusing on other, more easily measurable traits [42, 43].

The present study identified several traits that showed significant positive correlations with percentage survival. Therefore, it was possible to further investigate these traits using additional statistical methods to identify key attributes affecting yield. Consequently, path analysis was employed to gain deeper insights into the relationships between the studied traits and their impact on the percentage survival of Galbanum.

### Path Analysis

Stepwise regression analysis was conducted to identify the traits that most significantly influence survival percentage. The analysis began with plant dry matter content, which accounted for 26% of the variation. Next, leaf number was included, and together, these two traits explained a total of 33% of the variation in survival percentage (Tables 5 & 6). The resulting regression equation is:  $Y = -0.280 + 3.427 (\text{Plant dry matter content}) + 2.863 (\text{Leaf number})$ , where Y represents the survival percentage.

The result of the stepwise regression analysis was as follows (Fig 8):

$$Y = 27.56 + 3.71X_1 - 20.43X_2$$

In which Y is percentage survival, X1 is Plant dry matter content and X2 is leaf number.

Table 4 Stepwise regression for percentage survival Galbanum

Variables entered to model	Step	
	1	2
Intercept	12.142	-0.280
Plant dry matter content	3.232	3.427
Leaf number	-	2.863
R <sup>2</sup> (%)	26	33

Table 5 Direct (diagonal values) and indirect (values outside diagonal) effects of studied traits on percentage survival Galbanum

Trait	Plant dry matter content	Leaf number	Correlation with percentage survival
Plant dry matter content	0.540	-0.031	0.509
Leaf number	-0.061	0.274	0.213

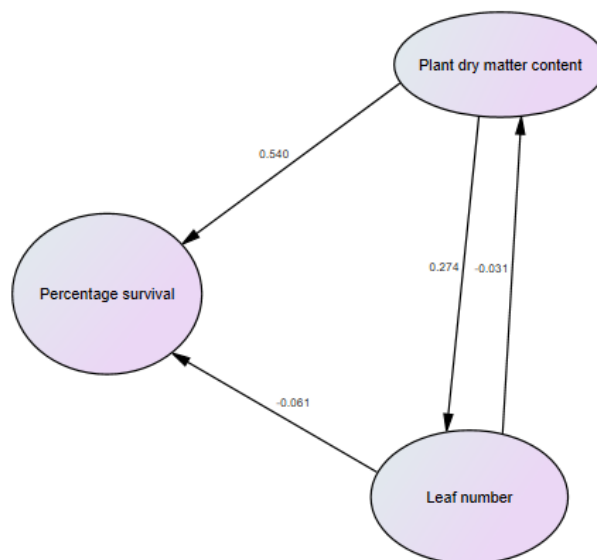


Fig. 8 Path analysis of studied traits on percentage survival Galbanum

## CONCLUSION

This research shows that the Galbanum plant thrives in low temperatures, particularly in May when temperatures are below 30°C and rainfall is abundant. Generally, primed seeds demonstrated superior growth and accumulated more reserves by the end of the growing season compared to other treatments. The combination of the earliest planting date, February 20, with primed seeds resulted in the highest tuber accumulation. Increased tuber weight enhances the chances of successful plant regrowth in subsequent years. Conversely, delaying the planting date reduces the plant's dry matter percentage. Therefore, for optimal Galbanum production, the recommended approach is to plant on February 20 using primed seeds.

## Conflict of Interests

There are none to declare.

## Ethics Approval and Consent to Participate

In the research, no harm was realized to animals or other persons.

## Consent for publications

Approval of the final manuscript is granted by all authors.

## Availability of Data and Material

Data are available on request from the authors.

## Authors' contributions

All authors shared the responsibilities in the design work analysis and design of the manuscript.

## Informed Consent

The authors declare not to use any patients in this research.

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