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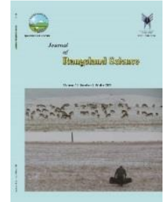

Research and Full Length Article:

Life Cycle and Phenological Growth Stages in Endangered *Fritillaria raddeana* Regel Using BBCH Scale in Its Natural Habitat, Northern Khorasan Province, Iran

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Abstract. Studying the phenology of valuable native plants which are on the verge of extinction, is very important and effective for better introduction and protection of them. *Fritillaria raddeana* is one of the native bulbous plants with ornamental and medicinal value in Iran. The aim of this study is to investigate the phenological stages of the *F. raddeana* as a first step to protect it and later to introduce it. In a natural habitat, in northern Khorasan province, Iran, six zones were selected at a distance of 1000 m from each other and five plants were selected and coded in each zone. To provide a standardized phenological description of the species, this study used the BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) scale. In total, from sprouting to winter rest, six main principal stages were described for sprouting, leaf development, inflorescence emergence, flowering, fruit development, fruit maturity, senescence and beginning of dormancy stages and within them, 15 secondary growth stages were described. Data were collected once a week in the course of the vegetative season and with a 30-day interval during the dormant season for two consecutive years (2015-2017). Additionally, we measured some traits of plant growth. During the first year, sprouting and subsequent leaf development began in late March and the inflorescence emerged in the middle of April. The full bloom occurred in the first half of May and then seed formation (in the late of May). The summer dormancy was triggered in late spring. During the second year of study (2016-2017), the results showed the growing stages start with a delay of 5-8 days compared with the previous year. In addition, T-test results showed the means of studied growth plant traits such as leaf number, plant height, flower stem length, flower number, and flower diameter were significantly higher in the first year than in the second one ($p \leq 0.05$). The differences of the phenology and studied variables between both years were related to different temperature and rainfall conditions. This narrow relationship with climatic conditions can imperil *F. raddeana* growth under the scenario of climate change.

Key words: Climatic change, Dormancy, Extinction, Native bulbous plants, Principal growth stage

Introduction

Fritillaria raddeana (Laleh Vajhegoon Gorgani *in Farsi*) (Liliaceae) is a perennial bulbous geophyte, native to Turkmenistan and north-eastern Iran (Brayan, 2002). In addition, this species can be promising for landscape design. Furthermore, this species is important as a source of biologically active substances due to its medicinal properties. Unfortunately, limited research has been done on various aspects of this species. In recent years, a variety of Iranian native *Fritillaria* species are at the risk of extinction due to their limited distribution and density, overgrazing, road construction, industrial emissions, illegal bulb and flower harvesting, flower smuggling and supply to the market, etc. (Farahmand and Nazari, 2015).

The weather and climate in general, have a profound impact on plants and affect various processes such as vegetation, flowering and phenology (Brickell, 2003). One of the most important effects of climate change is its impact on climate phenomena such as storms, droughts, heat waves and unexpected seasons (IPCC, 2007) which have profound effects on physical and biological processes at global and regional levels, on agriculture and natural resources in all their aspects (Allison *et al.*, 2009; Houghton *et al.*, 1992; Tegart *et al.*, 1990). One of the major impacts of climate change is the recent drought, which has greatly influenced agriculture and natural resources (Sabbaghpour, 2003).

The phenological study of native species in natural habitats is an important step for their preservation. Phenology is the study of plant and animal life cycle events, which are triggered by environmental changes. Thus, phenological events are ideal indicators of the impact of local and global climate changes on the earth's biosphere (Meier *et al.*, 2009). Phenology is one of the most important issues of ecology, investigating various stages of plant growth against living and non-living factors (Sakai *et al.*, 1999). The study of plant phenology is important for the better understanding of species biology, the management and conservation of ecosystems and establishment of landscape protected areas with the aim to domesticate them (Lesica and Kittelson, 2010).

Studying reproduction physiology and further domestication is of significant importance for conservation purposes and hopefully its commercialization in perspective. In general, temperature and rainfall greatly influence annual growth cycle of bulbous plants. Flowering in bulbous plants is affected by temperature cycles rather than light/dark cycles, therefore for these plants, the term “thermoperiodism” is mainly used instead of the term “photoperiodism” (Kamenetsky and Okubo, 2013).

Unfortunately, there are limited reports available on the study of the phenological stages of bulbous plants. Previous research showed some ornamental plants also have phenological growth stages such as *Rosa* sp. (Meier *et al.*, 2009), *Linum usitatissimum* L. (Smith and Froment, 1998), Saffron (Horacio Lopez-Corcoles *et al.*, 2015) and *Gladiolus* (Schwab *et al.*, 2015). Naghipour Borj *et al.* (2019) reported climate change is a major threat to natural habitats of *Fritillaria imperialis*. About 18% of *F. imperialis* habitats in central Zagros, Iran will be lost due to climate change by 2070 under two climate warming scenarios. Mahdavi *et al.* (2016) investigated the phenological stages of *Erythronium caucasicum* in two natural habitats on different altitudes varying from 110 to 650 m at sea level and reported that in the low-altitude area, growth cycles would begin about two months and half earlier. Shtein *et al.* (2016) reported that *Scilla hyacinthoides* life cycle is attuned to the annual rhythm, which alternates between hot and dry summer, cool and rainy winter. Zafarian *et al.* (2019) reported that *Fritillaria imperialis* began the sprouting (growth stage 0) after receiving 130.75 GDDs on March 23rd, 2016. The emergence of leaves began on 27th March with 166.30 GDDs and finished on 9th April. Gimdil *et al.* (2013) studied the phenology of *Allium hirtifolium* in Mashhad, Iran in relation to weather conditions and reported that the growth period of this bulbous plant was 150 days and flowering stage will begin after receiving 1187 heat units. Abtahi and Zandi Esfahan (2014) studied phenology of five range species in natural habitat and they reported that vegetative growth starts and continues until mid-May and the flowering stage lasted from mid-May to the end of June; afterwards, the seeding stage and seed dispersal have started.

Today, the situation of many natural habitats of bulbous plants, especially *Fritillaria*, is alarming in Iran and climatic changes have adversely affected growth and development of this plant species. Nevertheless, there are currently no universally used keys to describe the entire development cycle of this plant. Therefore, in this research, the extended BBCH (Biologische Bunde-sanstalt, Bundessortenamt und Chemische industrie) scale system can be useful for describing and recording the phenological growth stages of *F. raddeana* and for the standardization of phenology studies in this species. The aim of this study was to investigate the phenological growth stages of this endangered species for better understanding, management, and protection of its natural habitat and its establishment to domesticate it.

Materials and Methods

Study area

The studied natural habitat is located in the northern part of Khorasan province, Iran (Me'neh and Samalaqan County). The region is geographically located at 37°40′0″ N, 56°20′0″ E, at 1257 m a.s.l. (Fig. 1). This study was carried out using climatic statistics of the meteorological station, which is the closest to the natural habitat (Me'neh and Samalghan meteorological stations). The precipitation in this area includes both snowfall and rainfalls. This area has a short temperate spring and long cold winter. The duration of frost period is more than 110 days and the duration of the drought period is almost 3 months (Table 1). Soil characteristics were investigated and the results of soil analysis are presented in Table 1.

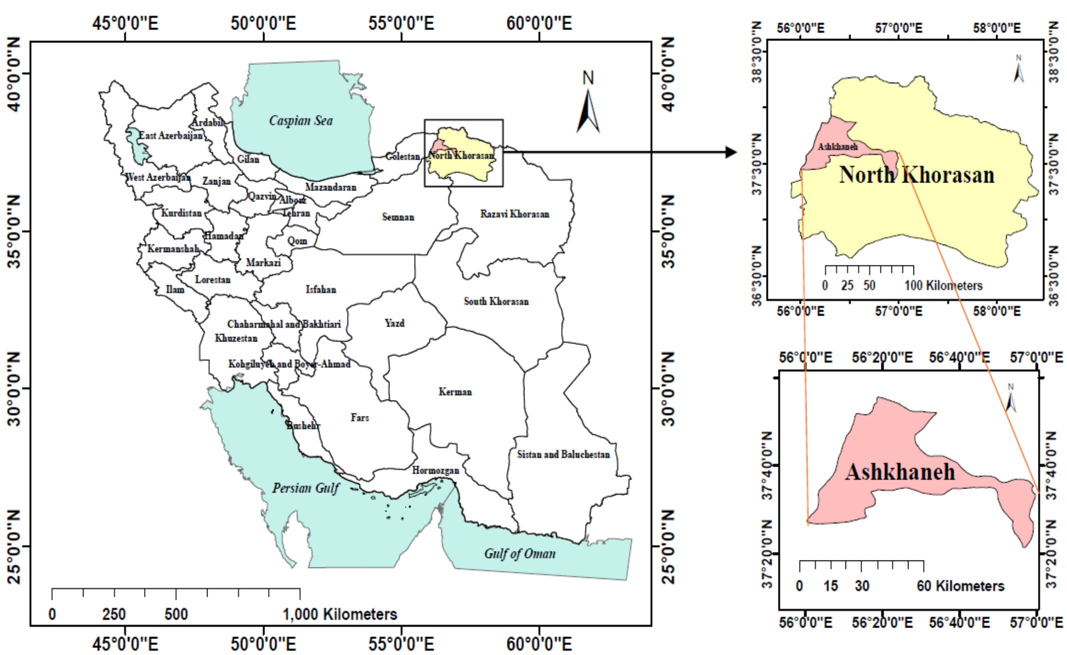

Fig. 1. Maps of location of the study area, North Khorasan Province, Maneh and Samalghan

Table 1. Averages of some climatic and soil characteristics of the study area

Climate features	Means	Soil characteristics	Means
Altitude (m)	1257	pH units	6.94
Average annual temperature (°C)	12.5	Electrical conductivity (ds/m)	0.37
Absolute minimum temperature (°C)	13-	Organic matter (%)	4.33
Absolute maximum temperature (°C)	30.9	Organic carbon (%)	2.51
Average annual rainfall (mm)	200	K mg/kg))	988
Average humidity (%)	40	P (mg/kg)	20
Freezing season (days)	110	N (%)	5.0
		Soil type	Silt Loam

Research method

In this study, phenological stages of *Fritillaria raddeana* were observed during two consecutive years from 2015-2016 to 2016-2017. First, the main natural habitat of *F. raddeana* was identified before the start of the study. Then, six plots were selected and five plants in each plot were marked. These plots were located more than 1000 m from one another.

In order to investigate and record the phenological stages of *F. raddeana* in natural habitats, seven principal growth stages of the plant cycle were selected according to BBCH scale as follows:

1- Sprouting (principal stage 0);
2- Leaf development (principal stage 1);
3- Inflorescence emergence (principal stage 5);
4- Flowering (principal stage 6);
5- Fruit development (principal stage 7);
6- Fruit maturity (principal stage 8);
7- Senescence and beginning of dormancy (principal stage 9)

The recorded data is presented in Table 2. The active growth stages of the plant (active growth of the stem) were monitored each week, and the stages of the inactive state (dormancy) each month. The important dates of different plant growth stages include: the bulb sprouting, the first leaf opening, opening of all the leaves, forming a flower stem, flower opening, drying the flowers, the blossom period, capsule forming, reaching the maximum size of the green capsule, seed maturation, beginning and end of the summer dormancy, the beginning of autumn growth, and the beginning and end of the winter rest.

Statistical analysis

During these two years, the plant growth characteristics, including leaf number per plant, number of leaves layers, plant height, stem diameter, flowering stem length, flower number per plant, petal length, flower diameter and peduncle length and diameter, were recorded that all of them comparison between two years based on independent *t*-test analysis.

Measurement of vegetative traits

When the plant reached its maximum size, final height and leaf number were measured. The diameter of flower, peduncle and stem were measured on the flowering stem. The summer dormancy began simultaneously with gradual changes in colour (yellowish) of seed capsule and drying aerial plant segments, and then ended late of summer when the roots of the plant restored their growth (Dehertogh and Le Nard, 1993). Data recording in autumn began when root formation starts. The onset of the winter rest is determined by decreasing the *Fritillaria* bulb temperature to below 10 °C.

The BBCH scale

The BBCH scale uses a two-digit numerical code: One digit for the principal growth stage (0–9) and another for the secondary growth stage (0–9) (Meier *et al.*, 2009). In this research, the principal growth stages correspond to the 10 main development stages of plant—i.e., sprouting (0), leaf development (1), inflorescence emergence (5), flowering (6), development of fruit (7), ripening and maturity of fruit (8), and senescence and beginning of dormancy (9). The secondary growth stages represent the percentage of growth accounted for, or duration of, each principal growth stage. The codes can be compared, and higher code numbers denote greater progression within the same principal stage (Table 2).

Table 2. Description of the phenological growth stages of *Fritillaria raddeana* according to the extended BBCH scale

BBCH Code	Description
Principal growth stage 0: sprouting	0 Bulb sprouting
Principal growth stage 1: leaf development	10 Opening first leaves, First pair of leaves visible 11 Opening full leaves
Principal growth stage 2: formation of side shoots (omitted)	
Principal growth stage 3: shoot development (omitted)	
Principal growth stage 4: development of harvestable vegetative parts (omitted)	
Principal growth stage 5: inflorescence emergence	51 Flower stem appearance, still enclosed by leaves 52 Flower bud's formation but without main color
Principal growth stage 6: Flowering	61 Blooming 62 Flower wilting
Principal growth stage 7: Development of fruit	71 Seed capsule formation 72 Maximum green capsule size
Principal growth stage 8: Ripening or maturity of fruit and seed	81 Wilting Seed capsule
Principal growth stage 9: Senescence, beginning of dormancy	91 beginning summer dormancies, shoot dead and dry 92 End summer dormancies, 93 Start Rooting, 94 End of Rooting, Bulb sprouting a little (1-2 cm) 95 Starting winter rest, stop sprouting and rooting 96 End winter rest, start bulb sprouting

Results

The results of this study showed that phenological development of *F. raddeana* had significant differences during the two studied years. For vegetative growth characteristics, *t*-test analysis showed a significant difference in leaf number per plant, plant height and stem diameter in two years at 1% level probability level (Table 3). The results revealed that the average leaf number per plant in the first year of

observation was more than second year. The average plant height in the second year was 15 cm less than in the first year (Table 3). In addition, the findings clearly indicated that the average stem diameter in the first year was 20% higher than the second year (Table 3).
All the flowering characteristics in this comparative study showed significant statistically differences between both years (Table 4). The average length of the stem during 2-year study was different, so that in the first year the flower stem length was 12 cm higher than in the second year (Table 4). Likewise, the number of flowers per plant in the first year was 30% higher than in the second year (Table 4). The comparison of means based on *t*-test showed that in the second year, the length of peduncle was 0.56 cm and flower diameter was 0.5 cm less than first year (Table 4). In general, *F. raddeana* had better growth performance and produced flowers with better quality in 2015-2016 than in 2016-2017.

Table 3. The comparison of mean vegetative growth characteristics of *Fritillaria raddeana* during two consecutive years based on independent *t*-test analysis

Year	Number of leaves layers	Number of leaves per plant	Stem height (cm)	Stem diameter (mm)
2016-2015	3.90	28.45	50.5	10.22
2017-2016	2.35	20.53	35.43	8.48
t-test	**	**	**	**

**= significant at 1% probability levels.

Table 4. The comparison of the mean flowering characteristics of *Fritillaria raddeana* during two consecutive years based on independent *t*-test analysis.

Year	Flower stem length (cm)	N0. flowers per plant	Petal length (cm)	Flower diameter (cm)	Pedicel Diameter (mm)	Pedicel length (cm)
2015-2016	42.5	8.10	3.45	3.12	2.53	3.44
2016-2017	30.5	6.25	2.22	2.66	2.20	2.88
t-Test	**	**	**	**	**	**

**= significant at 1% probability levels.

Phenological growth using BBCH scale

Stage 0: Sprouting

2015-2016: bulb sprouting occurred on March 20-25. At this time, soil and air temperatures were rising and the soil was sufficiently moist.
2016-2017: A review of this stage in the second year indicated that bulb sprouting appeared in the second decade of March (Table 5).

Stage 1: Leaf development

2015-2016: As presented in Table 5, first leaves were opened in the late of March, and in the first week of April, all leaves of the plant were opened.
2016-2017: A review of this stage in the second year indicated that first leaves appeared on March 20-23. All the leaves were opened in the last week of March. This stage was completed after 18 days (Table 5).

Stage 5: Inflorescence emergence

2015-2016: Observations showed that Inflorescence emerged in the firstten days of April in the pace with increasing temperature (Table 5).
2016-2017: inflorescence emerged between March 31st and April 4th (Table 5).

Stage 6: Flowering

2015-2016: Observations showed that the flowering period started from April 7-10 and lasted until May 8-17. Flower opening also occurred between the late of April and early of May. Flowering began with decreasing humidity and increasing the ambient temperature (air and soil). It should be noted that the flowering period lasted 8 to 12 days (Table 5).

2016-2017: the flowering stages of the *F. raddeana* began to grow seven days earlier in 2016-2017. The Flower wilting occurred in May 1 -11 and after that, *F. raddeana* entered to seed set stage. The flowering period in this year was between 10 and 14 days.

Stage 7: Development of fruit

2015-2016: The results of this year showed that seed capsules *of F. raddeana* formed on May 20-24. The green capsules reached the maximum size from late of May to early of June (Table 5).

2016-2017: The results showed that in this year, seed capsules were formed almost one week earlier compared to the previous year (Table 5). Seed capsules were formed on May 14 – 18 and after one week they reached its maximum size (May 20-25).

Stage 8: Ripening or maturity of fruit and seed

2015-2016: The results of this year showed the green capsules reached the maximum size from late May to early June (Table 5). Almost after 40 days from the formation of seed capsules, the seeds coat was completely dried and reached maturity.

2016-2017: The results showed that in this year, seed capsules reached its maximum size (May 20-25). Generally, the period from the capsule formation till the seed maturity was about 40 days.

Stage 9: Senescence, Beginning of dormancy

Summer Dormancy

2015-2016: *F. raddeana* began its summer dormancy after the middle of June (Table 5). During the summer, the bulbs were dormant and in the first half of September (September 4 to 16), their dormancy were finished. During dormancy period, the average of temperature ranged between 16 °C to 25 °C. In that year, the summer dormancy period was about 90 days (from June 15 to September 16).

2016-2017: Summer dormancy of bulbs was triggered in the first week of June 2016 (from June 4 to June 9). Compared to the previous year, the summer dormancy started 10 days earlier (Table 5). During dormancy period, the average of temperature was between 18 °C and 28 °C. In that year, the summer dormancy period was about 80 days (10 days shorter than the previous year).

Autumn Growth

2015-2016: Our findings demonstrated that bulbs start rooting from September 11. At this time, the days are short and the temperature is rather low (cool). This process continued until mid-October. At this stage, a number of white roots grow (emerged) and simultaneous bulb sprouting began (Table 5).

2016-2017: The process and dates of the rooting and bulb sprouting were quite similar to the previous year (Table 5).

Winter Rest

2015-2016: For the last part of October (October 22-28), the growth of bulbs slowed down, and they began their winter rest. In fact, with the reduction of daylight hours and the decrease in the air and soil temperatures, the bulbs entered the winter rest period. At this time, the temperature was below 4 °C. In March, when temperature and daylight hours were increased, the winter rest of the bulbs was interrupted (Table 5).

2016-2017: The winter rest began from mid-October (October 16). Same to previous year, this phase began when the days were shorter and the temperature was lower (Table 5).

Table 5. Phenological growth stages of *Fritillaria raddeana* in natural habitat during two successive years according to the BBCH scale.

2015 - 2016		**Plant growth stages**	**2016 – 2017**	
Principal growth stage 0 and 1 (sprouting and leaf development)				
Winter to Spring	March 20 to March 25	Bulb sprouting	March 12 to March18	Winter to Spring
	March 27 to March 30	Opening of first leaves	20 March to 23 March	
	Apri1 l to April 6	Opening of all leaves	25 March to 30 March	
Principal growth stage 5 and 6 (inflorescence emergence and flowering)				
	April 7 to April 10	Flower stem appearance	March 31 to April 4	
Spring	April 9 to April 15	Flower bud's formation	April 5 to April 11	Spring
	April 25 to May 4	Blooming	April 16 to April 27	
	May 8 to May 17	Flower wilting	May 1 to May 11	
	8 to 12 days	Average flowering period	10 to 14 day	
Principal growth stage 7 and 8 (development of fruit and Ripening or maturity of fruit and seed)				
Spring to Summer	May 20 to May 24	Seed capsule formation	May 14 to May 18	Spring to Summer
	May 27 to June 2	Maximum green capsule size	May 20 to May 25	
	June 17 to July 3	Wilting of seed capsule	June 9 to June 25	
Principal growth stage 9 (Senescence, beginning of dormancy)				
Spring to Summer	June 15 to June 19	Beginning	June 4 to June 9	Spring to Summer
	September 4 to September 16	End	September 5 to September 15	
Autumn growth				
Summer to Autumn	September 11 to October 5	Rooting start	September 10 to October 1	Summer to Autumn
	October 1 to October 15	End of Rooting	September 30 to October 14	
Winter rest				
Autumn to Winter	October 22 to October 28	Beginning	October 16 to October 26	Autumn to Winter
	March 7 to March 13	End	March 5 to March 10	

Growth cycle of *F. raddeana* in its natural habitats

The study of *F. raddeana* growth cycle in natural habitat revealed that the growth cycle of this plant, like other bulbous plants, is highly dependent on the temperature fluctuations (high and low temperatures). In early spring, bulb sprouting occurred with increasing temperature and depending on photoperiod. After the opening of all leaves, the flowering stem appeared. Typically, each inflorescence had more than three flowers. At flowering time, the temperature was cool. After flowering, the temperature was gradually increased and the seed capsule was formed.

Generally, seed capsules were large (2.5 cm length) and each capsule contained more than 100 seeds. From the late spring to early summer, temperatures rised and the bulbs start summer dormancy. In the summer, the average of temperature was above 27 °C, and the environment around the bulb had very little moisture since it does not rain. Our results showed that in mid-September, floral organogenesis occurred. This is the first report about floral organogenesis of *F. raddeana*. In the late summer and early autumn, the bulb rooting appeared when temperature lowered and daylight was reduced. With a decrease in temperature (below 10 °C), the plants gradually began their winter rest (Fig. 2).

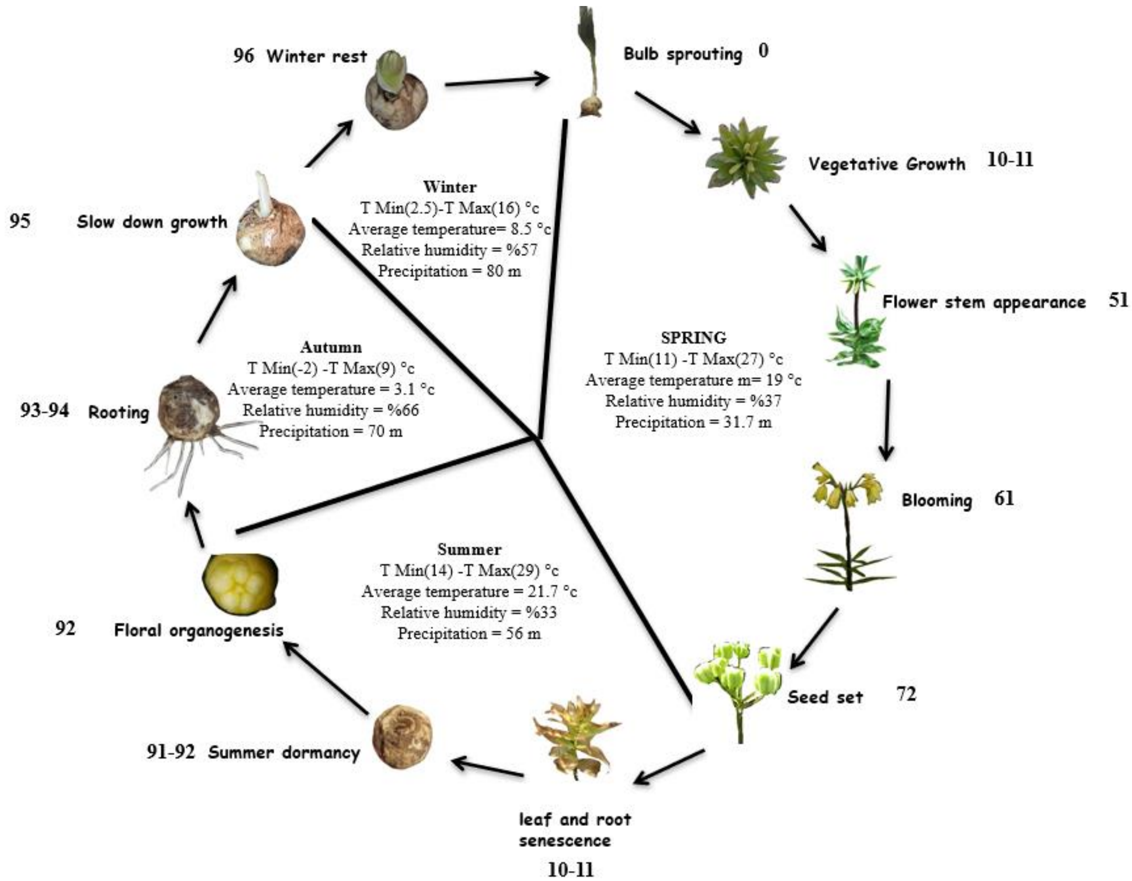

Fig. 2. Schematic representation of the annual cycle *Fritillaria raddeana* in natural habitat according to the BBCH scale (After Khodorova and Boitel-Conti, 2013)

The ombrothermic curve of study area over 18 years (2000 – 2017)

As shown in Fig. 3, Temperature and precipitation were fluctuating greatly over the 18 years. Excessive temperature changes and precipitation cause a serious warning for natural habitats of *F. raddeana*. As the figure shows, in late winter (February - March) the temperature gradually increases, and in early spring (March - April), during the vegetative growth season, the temperature was not warm and the rainfall was enough. During summer, in the summer dormancy season, the temperature was high and there was very little rainfall. In autumn, as the air cools, the rains begin gradually and it is necessary for the plant to grow in autumn. During the winter, the temperature was very low and the rainfall is high, which is necessary for the cold requirement of *F. raddeana*. As shown in Fig. 3, 4 and 5, the comparison of ombrothermic curve of the study area in the first year (2015-2016) and second-year (2015-2016) with the 18-years average showed the temperature trend in two years studied follows the 18-years pattern but the trend of precipitation was a little different (Fig. 3, 4 and 5).

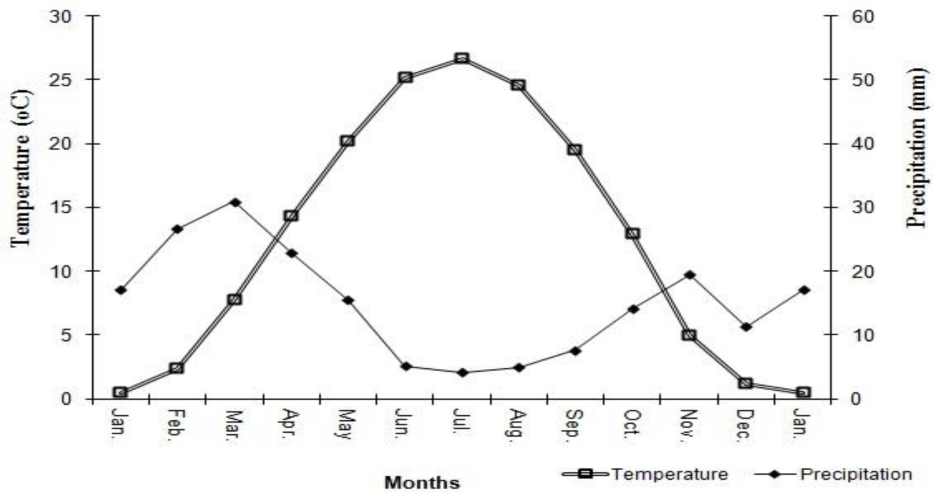

Fig. 3. The ombrothermic diagram of natural habitat of *Fritillaria raddeana* in 18 years (2000 – 2017)

Adaptation of phenological cycle of *F. raddeana* with the ombrothermic curve (2015-2016).

As shown in Fig. 4, the vegetative growth (stages 10-11) of *F. raddeana* began from March 20-25 and lasted till the first week of April. Results showed that sprouting (stage 0) started in late winter as soil and air temperature increased and also rainfall was abundant at this time (Fig. 4). Flowering stem of *F. raddeana* appeared from the beginning of the first decade of April, when temperatures were moderate and rainfall declined. During this flowering season, the flowering stages ended in the middle of May (Fig. 4). *F. raddeana* formed seed capsules in late May, the seeds reached full maturity by late August. In this period, the average of temperature was higher than 25 °C, and the precipitation was less than 5mm (Fig. 4).

Within the period of 2015-2016, the summer dormancy also began at the middle of June at the time when temperature rose and air was dry. Summer dormancy lasted till late summer, when temperature became cooler and daylight was reduced. In summer dormancy stage (July), air temperature was the highest of the year and the precipitation was about zero level (Fig. 4). In early October, the bulb started its rooting phase. Result showed in the middle of October, when temperature was below 12° C, and this period continued till the late winter when the temperature began to rise again. During the period of the winter_dormancy (Fig. 4), temperatures was lower and precipitation was higher than in other seasons (Fig. 4).

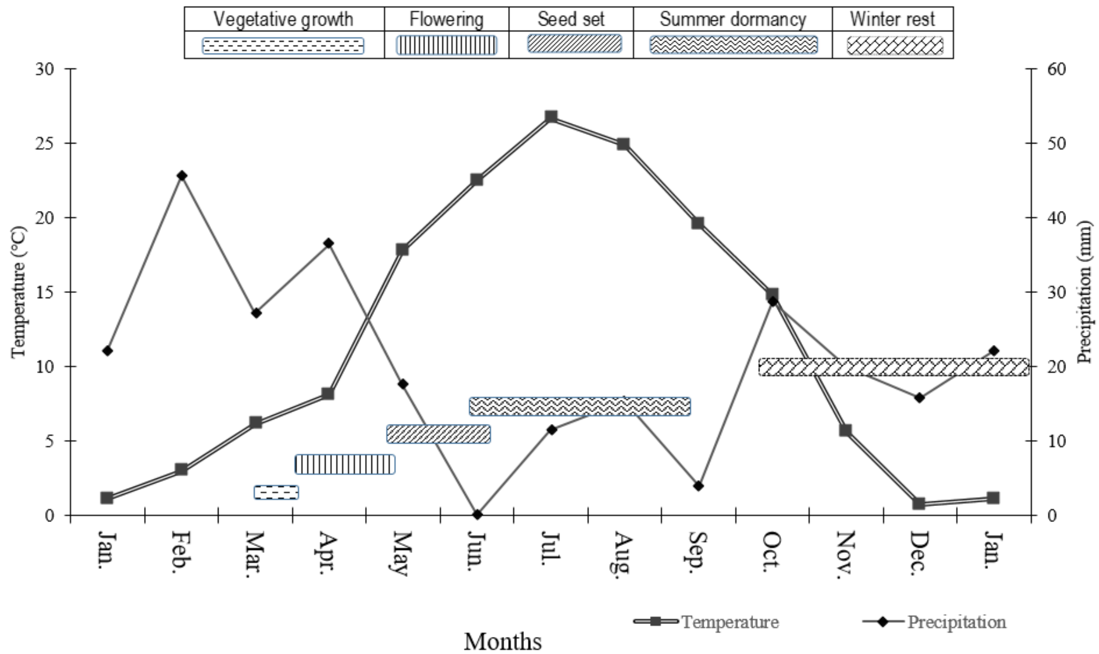

Fig. 4. The ombrothermic diagram and different phenological phases of *Fritillaria raddena* in 2015-2016

Adaptation of phenological cycle of *F. raddeana* with the ombrothermic curve (2016-2017)

As Fig. 5 shows, the vegetative growth of *F. raddeana* began in the middle of March and lasted till the late March. This stage began earlier than in the previous year. In the second year, it seems that air and soil temperatures were higher than in the previous year, which caused the earlier start of vegetative growth in the species (Fig. 5).

In this second year, the temperature was higher in the early spring than in the previous year, which led *F. raddeana* to begin its development earlier. It seems that the sudden increase in temperature causes a stressful condition to the plant. The flowering stem of *F. raddeana* has been observed since the beginning of late March to early April, when the temperature began to rise and rainfall decreases. Blossom of the species was observed over much of April and was completed at the late part of that month (Fig. 5). It started exactly one week earlier than in the last year (Table 5). In the second year, due to a sudden increase in temperature, from the middle of May, the capsules formed seeds, and by late June seeds reached full maturity (Fig. 5). At the time of seed capsule formation, the temperature of the air was increasing, but at the time of maturity, air temperature began to fall. No precipitation occurred during the formation of seed capsules until the stage of seed maturity (Fig. 5).

Observations showed that in the first ten days of June, when the plant was dry, the summer dormancy began and it continued till the late part of summer characterised by rather low temperatures and the reduction of daylight duration. During the summer dormancy, air temperature increased at the beginning of the period, but then showed a decreasing trend, at the same time precipitation was at zero level (Fig. 5). Since the end of September, when air temperature dropped and daylight hours shortened, *F. raddeana* entered the rooting phase. In late October, due to air temperature drop below 10°C, the species entered the stage of winter rest, and this period continued until late winter, when air temperature clearly showed an upward trend. In the period of the winter dormancy, temperature was lower and precipitation was higher than other seasons (Fig. 5).

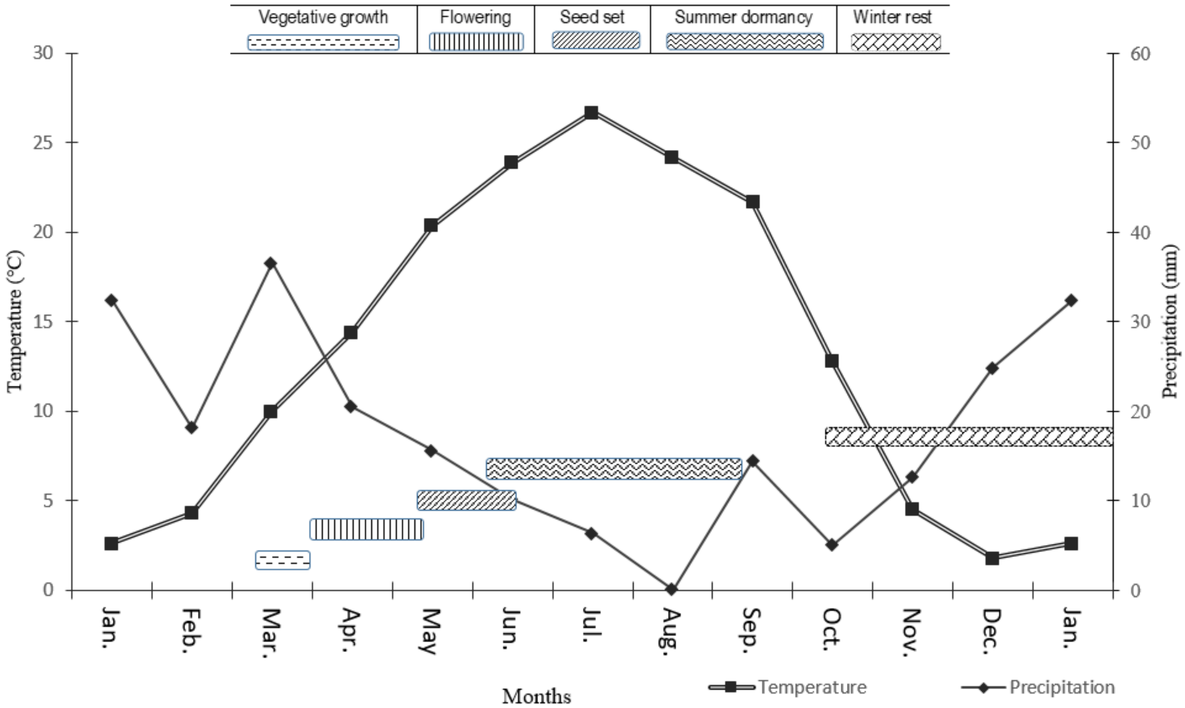

Fig. 5. The ombrothermic diagram (rainfall-temperature) and different phenological phases of *Fritillaria raddeana* in 2016-2017

As Fig. 6 shows, some important phenological growth stages of *Fritillaria raddeana* according to the BBCH scale photographed in its natural habitats (Fig. 6).

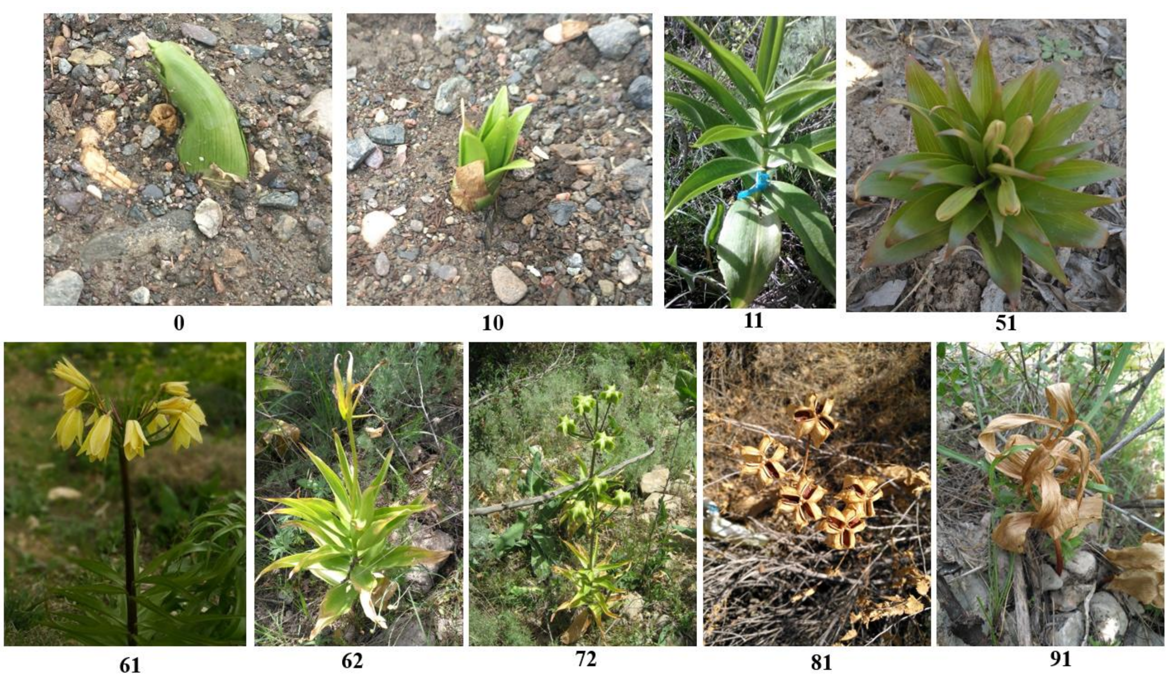

Fig. 6. Some important phenological growth stages of *Fritillaria raddeana* according to the BBCH scale.

Discussion

The initial phases of growth in plants are profoundly affected by local climatic conditions including temperature and rainfall (precipitation) (Brickell, 2003). *F. raddeana,* is a highly precious perennial bulbous plant due to its narrow and restricted natural habitat. The results of this study indicated that phenological growth stages of this bulbous plant are not fixed and vary from year to year. In this study, seven stages were considered for studying the phenology of this species as illustrated in the phenological graphs above. The investigation of phenological stages during two successive years, indicated that vegetative growth (growth stages 0 and 1) of this plant is significantly correlated with the level of winter rainfall. In 2015-2016 vegetative growth was delayed to March probably due to high snowing and low temperature in winter and early spring, the while in 2016-2017, because of high air temperature in winter, vegetative growth (sprouting stage) was observed before.

Vegetative growth in 2015-2016 continued until the middle of April (about 18 days) and, in 2016-2017 to early April (about 19 days), and taking into account air temperature, the emergence of flower stem in the first year (2015-2016), was observed from April 17th and in the second year (2016-2017) of observation from April 5th, i.e. 10 days earlier. Khodorova and Boitel-Conti (2013) reported, among several environmental factors, temperature is considered to play a predominant role in controlling proper growth and flowering in geophytes. They also reported, early spring plants start their aboveground development under particular ecological conditions, i.e., comparatively low air and soil temperatures and high insolation. Flowering (growth stage 6) in the first year (2015-2016) lasted to the middle of May and in the second year (2016-2017) it continued to the first ten days of May and seed set occurred in the middle-to-late of May. Seed maturation and dispersion are often synchronized in the second and third decades of July.

Investigating the phenological stages of *Erythronium caucasicum,* Mahdavi *et al.* (2016) reported that floral stalk emergence generally occurs in the middle-to-late of February. Salehi *et al.* (2016) studied phenological stages of *Iris fosterana* in its natural habitat in Northern Khorasan, Iran and reported that the growth period of this plant species started from late March and lasted to mid-July Investigation of the *Allium aschersonianum* growth life cycle showed bulbs of this species initiate inflorescence during the third year of their development from seeds, floral initiation occurs within the parent bulb in September, and flower formation continues for 2 months (Hovav *et al.*, 2001).

Investigation about the life cycle of *Allium aschersonianum* indicated that inflorescence initiation in this species occurs during the third year after seed propagation and floral initiation takes place within parent bulb in September. Flower formation lasts for 2 months, as well (Hovav *et al.*, 2001). Choon and Ding (2016) studied growth stages of *Etlingera elatior* and reported that it took 155 days from leafy shoot emerging from rhizome until senescence of inflorescence. The growth and development of torch ginger plant were divided into vegetative and reproductive phases. Study of phenological growth stages of saffron showed among all growth stages, vegetative growth stage is the longest period (142 days), followed by flowering (60 days), dormancy (55 days), reproductive (41 days), bud sprouting phase (36 days) and plant senescence (30 days) (Yasmin and Nehvi, 2018). Phenological stages of *Gladiolus* species from dormant corm to plant senescence, are divided into four developmental phases including dormancy phase, sprouting phase, vegetative phase (from emergence of the first leaf tip to emergence of the final leaf tip on the stem) and reproductive phase (from heading to plant senescence) (Schwab *et al.*, 2015).

The main point concerning the Iranian bulbous plants and particularly *F. raddeana* is its response to temperature and amount of rainfall especially during summer and winter rest (growth stage 9) which should be seriously accounted based on the available data from this study. In 2015-2016, precipitation was much higher than at an average during the summer rest (30 mm in summer season), resulting in adverse conditions for *F. raddeana* which is inactive during this period (summer rest). However, in the second year (2016-2017), the average of summer rainfall was lower than the previous year and consequently, a suitable condition for summer dormancy of *F. raddeana* bulbs. Most of bulbous plant require a “warm-cold-warm” sequence to complete their annual cycle (Khodorova and Boitel-Conti, 2013). Winter precipitation, preferably snow, is very important for further *Fritillaria* development because snow reduces soil temperatures fluctuation. As mentioned previously, in the first year that the winter precipitation was more abundant due to significant snowfall and minimal or no precipitation in summer, plant height was significantly higher (more than 50cm).

In most spring flowering bulbous plants if bulbs receive the needed amount of cold, its flowering quality will increase. In the genera of *Tulipa spp.* and *Fritillaria spp*., cold requirement has a direct and decisive role in flower stalk elongation and development of floral organs (De Hertogh and Le Nard, 1993). If hardening requirement is fully acquired by these plants, they produce normal flower stalk, otherwise, shorter and presumably abnormal flower stalks are produced (De Hertogh and Le Nard, 1993). Accordingly, it appears that for *F. raddeana*, winter and spring precipitation and the lack of summer rain is vital for its growth and development cycle. Considering the effects of temperature on the phenological shifts in *F. raddeana*, it should be pointed out that the reduction of winter precipitation and the lack of snow as well as higher temperature in the winter of 2016-2017, led to inadequate cold requirements, hence there were shorter flower stalks as presented in Table 4. Consequently, it could be assumed that good flowering (performance) in this species is deeply affected by low winter temperature as a prerequisite for cold requirements during the winter rest.

The findings of this study indicated that the phenology of *F. raddeana* is greatly influenced by climatic factors including temperature and precipitation, and each sudden and unpredictable change in these parameters affects various growth and developmental phases. Growth cycle of this species was different in the studied years indicating the unpredictable fluctuations in climatic conditions in its natural habitat. In the past years, the precipitation was generally as snow but in the recent years, because of global warming, precipitation is often seen as sporadic rain and results in phenological changes in growth cycle of this species. The natural growth cycle of this species is impaired under these unpredictable warming and this situation is ultimately harmful for this fragile bulbous species in its narrow habitat range. The results of this research revealed that based on environmental conditions such as air and soil temperatures, light intensity, orography, etc. and its place in habitat, the active growth cycle of this plant species is more than 40 days. Meanwhile, our investigations indicated that growth and development stages of this plant species within the studied area vary from year to year and temperature plays a key role in the start of each phase.

Regarding to the adaptability of this species to local region, it is necessary to pave the way for its acclimatization following its natural growth cycle. Since the habitats of many bulbous plants in Iran are challenging by many environmental and man-made factors (Farahmand and Nazari, 2015), we must firstly look at nature as a gene pool and prepare the ground for conservation these miracles and heritages of the nature particularly the critically endangered endemic ones. Only after this first step, we can involve them in floriculture industry through domestication process. This study as the first attempt to investigate closely this species within its natural habitats and could be considered as an important step in the introduction of *F. raddeana* as one of the invaluable and critically endangered endemic bulbous species in Iran.

Conclusion

As a result of our research about the phenological growth stages of *F. raddeana* for two consecutive years, we found that life cycle and phenological growth stages are highly dependent on climate characteristics such as temperature and precipitation. Consequently, any sudden change in these parameters causes changes in different growth stages of this plant. Only after a deep knowledge of the ecology of this species, we should domesticate it.

Therefore, the study of phenological growth stages of this endangered species can help researchers for better understanding, management, and protection of its natural habitat and its establishment to domesticate it.

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چرخه رشدی و مراحل رشد فنولوژیکی گیاه در معرض خطر انقراض لاله واژگون گرگانی *Fritillaria raddeana* در رویشگاه طبیعی آن در ایران بر اساس مقیاس BBCH

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چکیده. مطالعه فنولوژی گیاهان، به‌ویژه گیاهان بومی با ارزش و در معرض انقراض، برای معرفی بهتر و اهلی سازی این گیاهان و نیز حفظ ژرم پلاسم های آن‌ها بسیار مؤثر است. لاله واژگون گرگانی *(Fritillaria raddeana)* یکی از گیاهان پیازی بومی ایران بوده که ارزش زینتی و دارویی بالایی دارد. در رویشگاه طبیعی واقع در خراسان شمالی، ۶ ناحیه به فواصل ۱۰۰۰ متر از یکدیگر انتخاب و در هر ناحیه ۵ گیاه انتخاب و کدگذاری گردید. برای تهیه یک توصیف فنولوژیکی استاندارد از گونه ها، این مطالعه ابتدا از مقیاس BBCH (Biologische Bundesanstalt، Bundessortenamt und CHemische Industrie) استفاده شد. در مجموع، از جوانه زدن سوخ تا خواب زمستانه، شش مرحله اصلی رشد شامل جوانه زدن سوخ، رشد برگ، ظهور گل‌آذین، گلدهی، رشد میوه، بلوغ میوه، پیری و آغاز خواب و درون آن ها ۱۵ مرحله رشد ثانویه شرح داده شده است. داده‌های این پژوهش، هفته‌ای یک‌بار در طول فصل رویشی و با فاصله ۳۰ روزه در فصل خواب گیاه به مدت دوسال متوالی(۱۳۹۵-۱۳۹۷) جمع آوری گردید. علاوه بر این، برخی از صفات رشدی گیاه اندازه گیری شد. در سال اول، جوانه زدن و به دنبال آن رشد برگ در اواخر اسفندماه آغاز و گل‌آذین در اواخر فروردین‌ماه همان سال پدیدار شد. شکوفایی کامل گل‌ها در نیمه اول اردیبهشت ماه و سپس تشکیل بذر (در دهه اول خرداد) رخ داد. خواب تابستان در اواخر بهار آغاز شد. در طول سال دوم مطالعه (۱۳۹۷-۱۳۹۶)، گونه‌های گیاهی مورد مطالعه مراحل رشد را با تأخیر ۵-۸ روز نسبت به سال قبل نشان دادند. علاوه بر این، نتایج آزمون t نشان داد میانگین ویژگی های رشدی مطالعه شده مانند: تعداد برگ، ارتفاع گیاه، طول ساقه گل، تعداد گل و قطر گل در دو سال اختلاف معنی‌داری مشاهده شد ($p\leq 0.05$). تفاوت در فنولوژی و متغییر های مورد بررسی بین دو سال، با اختلاف درجه حرارت و بارش مرتبط بود. این رابطه تنگاتنگ با شرایط آب و هوایی، می تواند رشد لاله واژگون گرگانی *F. raddeana* را تحت تغییرات آب و هوا به مخاطره اندازد.

کلمات کلیدی: تغییرات اقلیم، خواب، در معرض خطر انقراض، گیاهان پیازی بومی، مرحله رشد اصلی