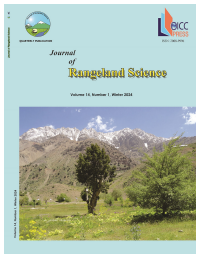

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Assessment of Phenological Stages of *Iris ferdowsii*, a New Endangered Species Based on BBCH System

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Abstract:

The basis of many plant studies is the identification of plant species because identifying plant populations in every region is the pivotal pillar of plants conservation. Furthermore, study of the stages of plant growth cycle is one of the ways to determine growth details of any given plant. The current research aimed to investigate the phenological stages of *Iris ferdowsii* in the main habitat of the plant based on the BBCH system for the first time (Biologische Bundesanstalt Bundessortenamt and Chemical Industry). The phenological study of *Iris ferdowsii* was performed during three consecutive years from 2018 to 2021. After selecting the habitat of this plant species, 10 different points of plant growth with a certain distance from each other were selected to study the different phenological stages of *Iris ferdowsii*. According to this system, the phenological stages of *Iris ferdowsii* was divided into 7 main stages and 13 sub-stages including: emergence, growth and development of two leaves, full growth and development of leaves, spathes stage, flowering, flower wilting, seed capsule formation, splitting of the seed capsule, the beginning of summer dormancy, the end of summer dormancy, the fall growth, the beginning of winter quiescence, and the end of winter quiescence. Identifying the new and endangered plant species of *Iris ferdowsii*, as a wild plant and a new genetic source provides researchers with the proper planning to preserve this species, domesticate and use it properly in breeding programs. We can also study its potentials in various ornamental and medicinal fields.

Keywords: Extinction; Flowering; Geophyte; Native plant; New plant

1. Introduction

Geophytes have an important place in ornamental plants, mainly due to the presence of underground stems, resistance against adverse environmental conditions, their use as an aromatic and medicinal plant, the ability of flowering in winter and early spring, the ornamental charm and the eye catching in the green space (Seyidoglu et al., 2009). The genus *Iris* L. is also one of the most diverse and popular genera in Iridaceae family (Mathew, 2001; Güner, 2012). In this context, *Iris ferdowsii* Joharchi & Memariani, *sp.* are the newest introduced species of this genus. It grows in gentle rocky slopes in the moderate or higher mountainous steppes of Hezar Masjed Mountains in northeastern Iran. Considering its geographical distribution, fragmented and isolated plant populations, and the reduction of suitable habitats for its growth, unfortunately, this plant species is in danger of extinction according to the IUCN Red List criteria and groupings. Furthermore, overgrazing, agricultural over-exploitation, and climate change have seriously threatened the life of this new plant species. Thus, in order to conserve its remaining population, necessary conservation measures in the main habitats of this plant as well as the outside are necessary. The name of this plant has been chosen in honor of the famous Persian poet and creator of Shahnameh "Ḥakīm Abul-Qâsem Ferdowsi Tusi" (Memariani, 2017). Endangered species are important genetic resources so that in the case of being extinct, the in-

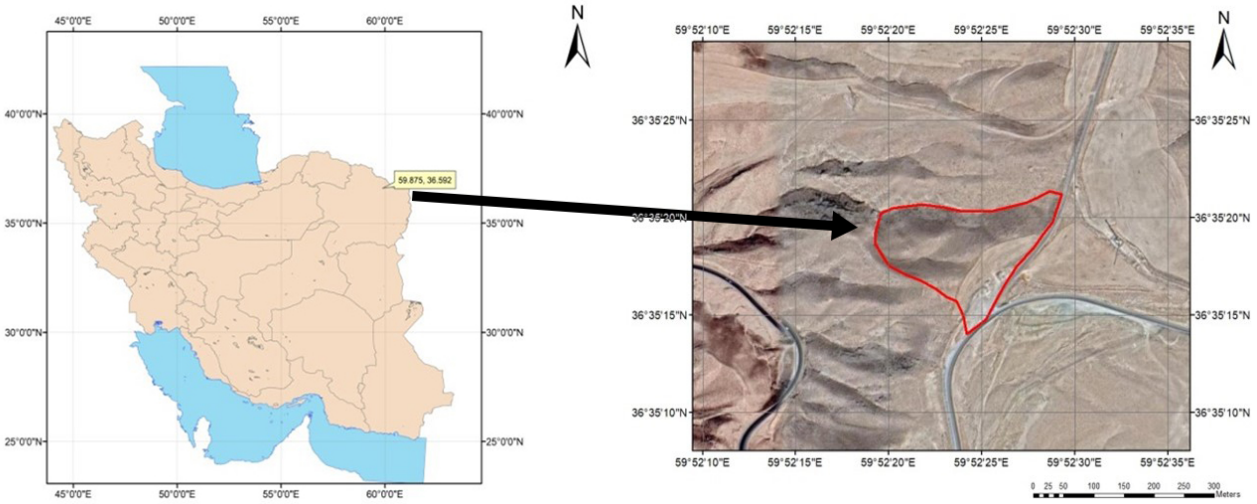


Figure 1. The study site of plant phenology

evitable outcome would be drastic changes in the ecosystem (Tohidifar and Haji Barat, 2017). Conservation of native plants of any country owing to the limited distribution of such plants, which increases their vulnerability to extinction, requires scientific and active studies (Naderi et al., 2017). Studying the stages of plant growth cycles is one of the ways to know and study the details of plant growth. Phenological responses of plants to environmental stimuli are species-specific so that they are strongly affected by climatic conditions (Wolkovich et al., 2012; Chuine and Régnière, 2017). Improving scientific understanding of the relationship between phenology and the structure and function of ecosystems contributes to the awareness of natural resources adaptation management (Pacifici et al., 2015). The study of the phenology or the plant's growth cycle's periods such as bud burst, flowering, and fruiting affects different levels of biological processes on both the individual and the ecosystem. In addition to affecting each plant individually, phenology also influences the growth of the organisms that depend on it. (Anderson et al., 2012; Miller-Struttmann et al., 2015). Phenological changes cause marked modifications in plant growth, reproduction, coexistence of different plant species and pollinators (Sun and Frelich, 2011). The developed BBCH classification system is a uniform system for coding different phenological growth stages in all monocotyledonous or dicotyledonous plants, which has been expressed and implemented by Enz and Dachler (1997). This method is widely used for phenological studies in agriculture, horticulture, environment and climate fields (Meier et al., 2009). Phenological study of *Fritillaria raddeana* Regel based on BBCH system in two consecutive years showed that climate changes during two years have impacted the growth traits in the plant so that vegetative and reproductive traits were affected by the changes in rainfall and temperature (Alipour et al., 2021). Phenological study of *Crocus sativus* L. by BBCH method revealed six main growth stages: starting from sprouting, cataphylls and flowers appearance, plant appearance and development, replacement corms development, plant senescence and corm dormancy (Lopez-Corcoles et al., 2015). Plant phenology in *Sapindus mukorossi* was carried out based on BBCH classification system and 8 main growth stages were determined: bud development, leaf development and shoot development, inflorescence emergence, flowering, fruit development, fruit maturity and seed, senescence and beginning of dormancy. Also, 58 secondary growth stages were identified (Zhao et al., 2019). Elimination of key species from natural habitat leads to the increase of environmental crises, making the life difficult for future generations. Therefore, access to practical information to preserve these valuable genetic resources and ultimately, their use in breeding programs and cultivation development are the main objectives of the researches examining ecological characteristics of plant species in their natural habitats (Oshib Nataj et al., 2011). Taken together, the present study was conducted to assess the possibility of conserving the endangered plant species of *Iris ferdowsii*, as one of the new plant genetic resources, by studying different growth stages of the plant in its main habitat.

2. Materials and methods

2.1 Experimental site

In order to study the phenology of *Iris ferdowsii*, the habitat with the highest number of *Iris ferdowsii* was selected. The place was located in the northeast of Iran, Khorasan Razavi province and at a distance of 60 km from Mashhad-Kalat road (Fig. 1). The soil characteristics of the study area are listed in Table 1.

2.2 Observations of phenological stages based on BBCH

The phenological study of *Iris ferdowsii* was performed during three consecutive years from 2018 to 2021. After selecting the habitat of this plant species, 10 different points of plant growth with a certain distance from each other (250 m) were selected in order to study the different phenological stages of *Iris ferdowsii*. In the BBCH classification system, the different growth stages are distinguished from each other by coding method (Table 2).

Table 1. Soil characteristics of the study site (Natural habitat of *Iris ferdowsii*)

Texture	Silt (%)	Sand (%)	Clay (%)	EC (dS/m)	Soil pH	Potassium (mg/kg)	Phosphorus (mg/kg)	Nitrogen (mg/kg)	Organic carbon (%)
Loam	31	50	19	3.98	7.67	203.3	5.1	560	1.31

3. Results

3.1 Principal growth stage 0: Sprouting

This stage includes germination of rhizome and commence of plant growth (00) (Fig. 3). After the completion of winter quiescence in plant and the increase of temperature, plant growth starts and plant leaves grow (Fig. 2). Phenological studies during three years showed that growing of rhizome occurred in March and varied considering the climatic changes and precipitation from early to mid-March (Table 3).

3.2 Principal growth stage 1: Leaf development

In this stage, leaf growth and development were studied in two stages. In the first stage (11) (Fig. 3), growing time of two completely developed leaves was registered. The results showed that emergence of two completely developed leaves in plant occurred from mid to the end of March. In the next stage (14) (Fig. 3), growth and development time of plant leaves was investigated. In the first year, growth and development of leaves started from the end of March and continued until the early April. In the second and third years, growth and development of leaves were observed in the first half of April.

3.3 Principal growth stage 5: Inflorescence emergence

The phenological stages of flowering in the plant were also examined when the plant entered the reproductive growth stage. At this stage of growth, the emergence time of flower buds (55) (Fig. 3) in the plant was examined, and the results showed that flower buds in the plant emerged in April (Fig. 2). In the first year, the emergence of flower buds was observed in the first half of April. In the second and third years, flower bud emergence was recorded in the second half of April (Table 3).

3.4 Principal growth stage 6: Flowering

This stage of plant growth includes the flowering time of the plant. Assessing the phenological stages in the plant revealed that flowering (66) (Fig. 3) in the first year occurred in the last week of April while it occurred in the first half of May and in the first week of May in the second and third years, respectively (Table 3). After wilting of plant petals, flower wilting stage was recorded in the plant (69) (Fig. 3). It was also observed that flower wilting in the plant in the first year was observed in the first week of May and in the middle of May in the second and third years (Table 3).

3.5 Principal growth stage 7: Development of fruit

The time of seed capsule formation in the plant was assessed at this stage of growth (77) (Fig. 3). Seed capsules were formed after wilting of flowers in May (Fig. 2). Seed capsules were formed in the first week of May in the first year, and in the third week of May in the second and third years (Table 3).

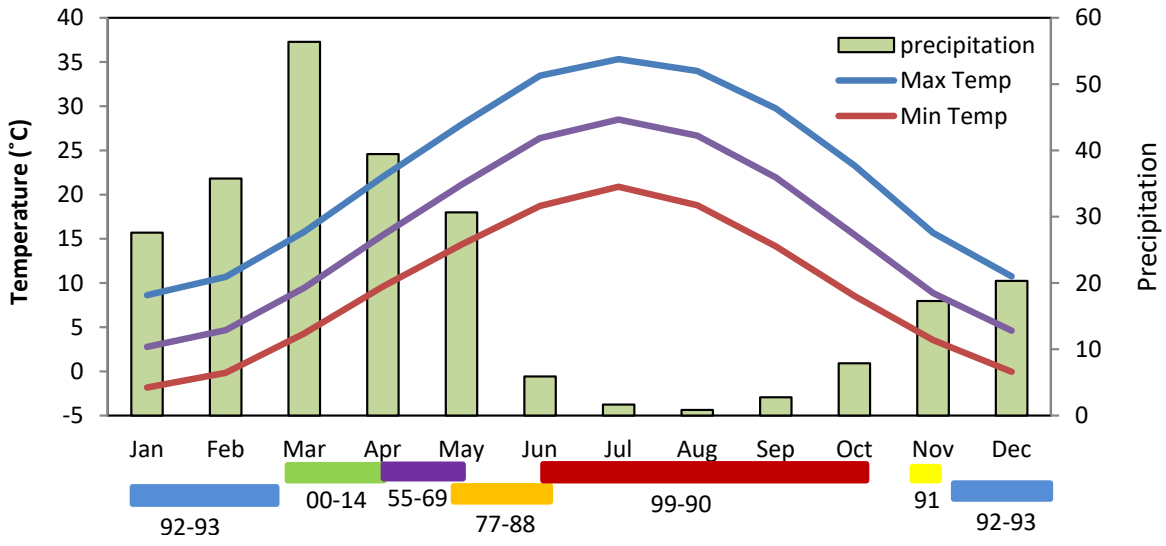


Figure 2. Different phenological stages of *Iris ferdowsii* based on BBCH scale. Mean temperature, Maximum temperature, minimum temperature and precipitation are based on thirty-year average from 1990 to 2020

00

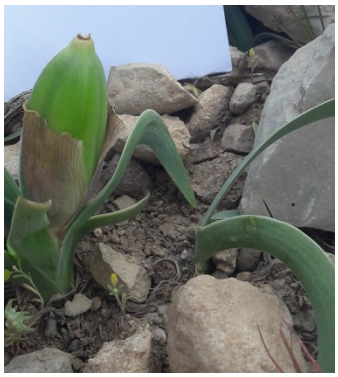
11

14

55

66

69



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88

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90

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92

Figure 3. Different phenological growth stages observed in *Iris ferdowsii* classified according to BBCH scale. 00: Sprouting; 11: Opening of two leaves; 14: Opening of full leaves; 55: Spathes stage; 66: Full bloom; 69: End of flowering; 77: Seed capsule formation; 88: Wilting Seed capsule; 99: Beginning of summer dormancy; 90: End of summer dormancy; 91: Autumn growth; 92: Beginning of winter quiescence

Table 2. Description of the phenological stages of *Iris ferdowsii* based on the BBCH universal scale

BBCH scale	Phenological stage description
Principal growth stage 0: Sprouting	
00	Sprouting
Principal growth stage 1: Leaf development	
11	Opening of two leaves
14	Opening of full leaves
Principal growth stage 2: Formation of side shoot (omitted)	–
Principal growth stage 3: Stem elongation or rosette growth (omitted)	–
Principal growth stage 4: Development of harvestable vegetative parts (omitted)	–
Principal growth stage 5: Inflorescence emergence	
55	Spathes stage
Principal growth stage 6: Flowering	
66	Full bloom
69	End of flowering
Principal growth stage 7: Development of fruit	
77	Seed capsule formation
Principal growth stage 8: Ripening or maturity of seed	
88	Wilting Seed capsule
Principal growth stage 9: Senescence, beginning of dormancy	
99	Beginning of summer dormancy
90	End of summer dormancy
91	Autumn growth
92	Beginning of winter quiescence
93	End of winter quiescence

3.6 Principal growth stage 8: Ripening or maturity of seed

At this stage, seed maturity growth was considered at the same time as opening of seed capsule (88) (Fig. 3). Seed ripening and splitting of the seed capsule in the first year occurred in the first half of July, in the second half of July in the second year, and in mid-July in the third year (Table 3).

3.7 Principal growth stage 9: Senescence and beginning of dormancy

After examining the phenological stages of the plant, it was found that summer dormancy began after flowering and along with increasing temperature and decreasing rainfall and the beginning of summer (Fig. 2). This stage began with the complete drying of the plant leaves (99) (Fig. 3). In the first year of the study of plant growth stages, summer dormancy and complete drying of leaves began in mid-July. In the second and third years, it was found that the onset of summer dormancy and complete drying of the leaves began in late July. After the end of summer season and with the decrease of temperature and the beginning of autumn rains, summer dormancy in the plant ended (90) (Fig. 3). Autumn growth in the plant was expressed by the appearance of leaf tips on the soil surface (91) (Fig. 3). According to the phenological stages in the plant, it was found that autumn growth in the plant occurred in November. In the first, second and third years, the autumn growth of plants began on November 10th, 6th and 1st, respectively. As the temperature decreased in late autumn and early winter, plant growth ceased and plant entered a dormant period (92) (Fig. 3). Winter quiescence in the plant began in late November and lasted until March when the temperature rose again (Fig. 2). The end of winter quiescence in plant was considered at the same time as the emergence of rhizomes in the plant. With increasing the temperature, the leaves of the plant resumed their growth and the growth of the plant began (Table 3).

4. Discussion

Seasonal variations are an integral part of earth depending on how it is positioned and rotated. In temperate regions and northern latitudes, plants use photoperiod, vernalization and day-growth degree to approximately synchronize flowering and other activities with suitable environmental conditions (Wright et al., 2019). In recent decades, climatic changes have led to phenological changes in plants (Fu et al., 2015). In this study, different phenological stages of *Iris ferdowsii*

Table 3. Different growth stages of *Iris ferdowsii* in consecutive three years

Plant growth stages	2018 – 2019	2019 – 2020	2020 – 2021
Principal growth stage 0: Sprouting			
00: Sprouting	4 Mar – 8 Mar	6 Mar – 10 Mar	8 Mar – 13 Mar
Principal growth stage 1: Leaf development			
11: Opening of two leaves	13 Mar – 23 Mar	17 Mar – 27 Mar	23 Mar – 30 Mar
14: Opening of full leaves	25 Mar – 3Apr	1 Apr – 14 Apr	2 Apr – 10 Apr
Principal growth stage 5: Inflorescence emergence			
55: Spathes stage	6 Apr – 15 Apr	17 Apr – 26 Apr	13 Apr – 19 Apr
Principal growth stage 6: Flowering			
66: Full bloom	20 Apr – 28 Apr	1 May – 10 May	30 Apr – 7 May
69: End of flowering	1 May – 4 May	12 May – 15 May	11 May – 14 May
Principal growth stage 7: Development of fruit			
77: Seed capsule formation	3 May – 8 May	15 May – 22 May	16 May – 21 May
Principal growth stage 8: Ripening or maturity of seed			
88: Maturity of seed	10 Jun – 15 Jun	20 Jun – 27 Jun	15 Jun – 22 Jun
Principal growth stage 9: Senescence and beginning of dormancy			
99: Beginning of summer dormancy	16 Jun – 21 Jun	28 Jun – 2 Jul	23 Jun – 28 Jun
90: End of summer dormancy	18 Oct – 26 Oct	20 Oct – 27 Oct	11 Oct – 17 Oct
91: Beginning of autumn growth	10 Nov – 15 Nov	6 Nov – 14 Nov	1 Nov – 9 Nov
92: Beginning of winter quiescence	27 Nov – 2 Dec	21 Nov – 26 Nov	21 Nov – 28 Nov
93: End of winter quiescence	6 Mar – 10 Mar	8 Mar – 13 Mar	10 Mar – 14 Mar

plant based on BBCH standard classification system were studied for the first time in three consecutive years. The results showed that in the three years of study, changes of temperature and decrease or increase in rainfall affected different stages of plant growth. According to the observations made during these three years, it was determined that flowering and flower quality in the second and third years were affected by changes in rainfall and temperature, and that the amount and time of seed production in the plant varied based on these changes. In comparison with other environmental factors, temperature has a decisive role in the flowering of geophytes (Khodorova and Boitel-Conti, 2013), and is one of the main and influential factors in the beginning of each stage of plant phenology, both vegetative and reproductive (Rosenzweig et al., 2008; Zafarian et al., 2019). In the second year, based on the amount of rainfall and temperature difference compared to the first year, and according to the observational data, the number and quality of flowers produced were significantly different from those of the first year. The main stages of phenology including leaf drop, leaf flushing, flowering and fruiting were strongly affected by the water available to the plant. The stages of leaf flushing, flowering, and fruit growth and development begin by the rainy season while plant foliage fall in most species begins with the onset of the dry season (Do et al., 2005; Singh and Kushwaha, 2006; Valdez-Hernández, 2015). Similarly, in *Iris ferdowsii*, yellowing of leaves began at the same time as the beginning of the warm season and the end of the rainy season. Also, the beginning of regrowth and flowering in the plant was simultaneous with the onset of rainfall and reduction of air temperature. Changes in the phenological rotation of different plant species due to environmental changes lead to changes at the community level (Boose et al., 2003).

5. Conclusion

In the present study, for the first time, different stages of vegetative and reproductive growth of the new *Iris ferdowsii* plant were described using the global BBCH scale. Plant phenological observations were performed for three consecutive years. This led to accurate recording of different vegetative and reproductive stages of the plant in different temperature conditions of the region and provided comprehensive information on plant growth for further studies. Awareness of the environmental conditions of new ornamental plants allows, in addition to increasing the basic information regarding the preservation of these plants in the growing habitat and outside the main habitat, to be used as new genetic resources in other studies including plant breeding. It is also possible to

domesticate these valuable plants in order to use them in the ornamental plants industry. Increased population growth, climate changes and habitat destruction due to overgrazing, road construction and plant collection by local people are among the factors that have led to the extinction of plant species. This has reduced biodiversity, which is a very serious threat to the environment. As a result, collecting and increasing information about new plant species and introducing them are among the main and practical strategies in conserving these valuable genetic resources. Our research shows that unfortunately, due to the worrying conditions of this species such as small natural habitat area, very small population, location the habitat on private agricultural land and most importantly climate change, this species is in a warning state and if it is not protected, it will become extinct in the future years. Therefore, the results of this study as the first research work on *Iris ferdowsii* can be an important step in preserving and domesticating this valuable and endangered species.

Ethical approval:
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval does not applicable.

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Authors Contributions:
All authors have contributed equally to prepare the paper.

Availability of data and materials:
The data that support the findings of this study are available on request from the corresponding author.

Conflict of Interests:
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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