Biology of *Crocus olivieri* subsp. *olivieri*

Hasan Vurdu, Kerim Güney and Fatma F. Çiçek
Gazi University, Faculty of Forestry
Kastamonu, 37200,
Turkey

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Abstract

Phenological periods and morphological characteristics of *Crocus olivieri* subsp. *olivieri* have been determined by observations in the sampling area. The changes in morphometric characteristics of this species for a period of 5 months from flowering to leaf disappearing have been recorded. The corm germination is achieved in 50±18 days under indoor conditions. 1000 seed weight has been determined as 6.819 g and the viability of seeds ranged from 89 to 96 percent. Different seed germination procedures were tested. Among these, seeds kept in distilled water for 1-10 days showed to be the suitable for the seed germination. Germination of the seeds started 154-179 days after sowing. As a result, the cultivation of this species could be done commercially by using corms and seeds.

INTRODUCTION

Crocus species are members of *Iridaceae* family. The plants in this family are herbs with rhizomes, corms or bulbs (Davis, 1984). The family Iridaceae embraces about 60 genera and 1500 species. The genus *Crocus* embraces about 70 species distributed mostly in the Mediterranean Region (Warburg, 1957). In Turkey, there are about 32 species of this genus (Malyer, 1985). Among these species, *Crocus olivieri* Gay. (Figure 1) is distributed in western, northern, southern and Central Anatolia and Greece, southern Yugoslavia, southern Bulgaria, southeastern Romania and Albania. A well-known *Crocus* is *C. sativus* L. (Figure 2) that is cultivated on a small scale in Mediterranean and Asiatic countries.

The plants in the genus *Crocus* have underground fleshy corms. Their corms are usually symmetrical and enclosed by several fibrous, membranous or coriaceous tunics. They have basal, grass-like, dark green leaves with whitish median stripe. On the lower surface of the leaves, there are two deep grooves on either site of flattish keel. Their leaves appear with flowers or after flower. Depending on the *Crocus* species, the flowers appear in spring or autumn. A plant may have one or several flowers. Each flower is on a short subterranean pedicel that is subtended by a membranous sheating prophyll. The flower has a membraneous bract. The perianth tube is long and narrow. The flower has six segments in two whorls. The style of the flower is three or more branched. Ovary of these plants is subterranean. The fruit is a capsule and they have numerous seeds with brownish or reddish color. Species of *Crocus* can be identified from each other by several features. The styles of the *Crocus* vary greatly from species to species. They can be dissected into three lobes or very finely dissected with numerous lobes. The character of corm tunic is another important identification feature. There are corm tunics with circular rings around the entire corm, with fibers that run parallel from top to bottom, with overlapping scales as shell, with finely or coarsely reticulate. Other characteristics that aid in the identification of species are appearance of foliage before or after flowering, number of leaves, the flower color, and the anther color (Bryan, 1989). Most *Crocus* species can be propagated either by annual replacement corms or by seeds. Due to sterility, a few species of *Crocus* are propagated solely by replacement corms (Negbi et al., 1989; Plessner et al., 1989), such as *C. sativus* (a triploid species) (Chichiricco, 1984; Ghaffari, 1985). Most of the *Crocus* species grow naturally in the fields between shrubs and grass or light woodlands (Huxley, 1975). Very limited numbers of *Crocus* species are being cultivated. These are mostly *C. sativus, C. aureus* and *C. vernus.*

The aim of this study was to examine the biological and morphological characteristics of *C. olivieri* in order to find out cultivation techniques for this plant.

MATERIALS AND METHODS

Description of Study Area

The southern region of METU Campus in Ankara, Turkey, was selected as sampling area. The sample area is covered with *Pinus nigra* trees. *C. olivieri* grows between rows of pine trees in the forest where they can get the direct sunlight mostly at noon. Within the sampling area, the specific study areas were located on the basis of high population density of *C. olivieri.*

Sampling Guidelines

Since flowering ends in a short period of time, and leaves disappear during summer, labeling of the plants has a significant importance to relocate the sample plants in the field. The labeling procedure was carried out in early April 1992, when *C. olivieri* dominates the flora. The locations of plants on the study area were labeled by 2x2x40 cm sized sticks (Figure 3). The sticks were inserted western side of each plant with a 7 cm apart. Each stick was numbered. A total of 400 plants were labeled for the study (Figure 4).

Data Collection on Phenology of the Species

Changes in the growth and development of *C. olivieri* in a year around were observed. Phenological characteristics such as sprouting, leaf appearing, flowering, fruit maturation and leaf disappearing time of the sample plants were recorded monthly. These characteristics, as used in this study, are defined as follows. Sprouting time is the time at which corms were started to give bud. Leaf appearing time is the time at which leaf of the plant is seen on the ground. Flowering time is the time at which most of the plants in the field are flowering. Fruit maturation time is the time at which seeds were spread on the soil after opening of the capsule. Leaf disappearing time is the time at which most of the leaves become dry (Anonymous, 1992).

Data Collection on Morphology

Every month, 30 plants were collected from the sampling area and brought to the laboratory. On these collected samples, morphological characteristics were recorded and the morphometric characters were measured. Descriptions of these characters and their abbreviations are shown in Figure 5. The longer one of the two leaves and flowers of each plant is considered during leaf and flower length measurements. Morphological characteristics such as color and size of *C. olivieri* growing in the study area were also determined. In addition, 1000 seed weight was also determined.

Corm Propagation

Germination studies of corms were carried out in sand pots. 62 *C. olivieri* corms were stored and kept in nylon bags at 4 ^{o}C for germination studies. Corms were planted at a depth of 10 cm, in rows 5 cm apart and at a distance of 5 cm within the rows in seven pots. 20 corms were planted in two sand pots in the laboratory immediately after collection from the field and watered regularly.

Seeds

Before the seed germination tests, the viability of seeds determined. The viability of *C. olivieri* seeds was tested by using two different methods.

Biochemical Test (Tetrazolium Test)

The tetrazolium test is a biochemical method in which viability of seeds is tested by the red color appearing when the seeds are soaked in 1 % 2,3,5 triphenyl tetrazolium

chloride solution prepared by dissolving 10 g of 2,3,5 triphenyl tetrazolium chloride in 1000 ml distilled water (pH=6.5-7.0). Seeds were prepared by cutting at the end that is the opposite of the radicle and they were immersed in distilled water for 20 hours. Then, seeds were cut longitudinally for exposing embryo to tetrazolium. After cutting, they were transferred into 2,3,5 triphenyl tetrazolium chloride solution for 20 hours. Completely red colored embryos indicate viable seed (Anonymous, 1986; Hartmann and Kester, 1968).

Viable Seed Testing (Germination Percentage)

200 seeds were selected at random. The seeds were kept in water for 24 hours, then in humid sand/perlite pot and stored at room temperature. Since the germination period for seeds has not been determined yet, the time that seeds started to germinate was recorded as first germination data. Until seeds completed their germination, germinated individuals were counted. as data for viable seed testing (Anonymous, 1979).

Seed Germination Tests

Seeds were collected in the field in June just before the capsules opened and the seeds fell on the ground. After weight measurements, they were stored at 4 ^{o}C in brown paper bags. To determine the most suitable seed germination procedure, different seed germination alternatives were examined (Çiçek, 1994).

RESULTS

Phenological Characteristics

As a length of phenological periods, sprouting continued from early October to next March. Leaves appeared with flowers on March. After 21 days, flowering period ended. Leaves started to dry in early August, then, disappeared. *C. olivieri* fruits began to develop in March and maturated in early August. Seeds spread on the soil after opening of the capsule in August. The length of these phenological periods was given in Table 1. The phenological periods according to months were also demonstrated graphically in Figure 6. At the laboratory conditions, sprouting began in September, flowering took place in late December.

Morphological Characteristics

C. olivieri is a perennial, cormous, herbaceous plant. Its flowering continues from late March to late April in a period of 21 days. Its flower color ranges from pale lemon yellow to deep orange. *C. olivieri* has a fibrous root system. Its corm is enclosed by a brown tunic that is membranous, splitting at the base into parallel fibers and at the apex into sharp fibrous points as shown in Figure 7.

There are mostly two leaves, which are often slightly spreading rather than erect on each plant. A single leaf or three leaves are also observed. Leaves are deep shiny green, rather broad, 2-5 mm wide and have a white stripe in the middle. Leaves appear with flowers (synanthous). In flowering season, leaves are either slightly shorter or slightly longer than the flowers. Afterwards, they continue to grow until maturation that is about 40 cm in length. Then, they start to dry. One or two flowers are observed on each plant perianth of the flower is 5-7 cm long and has 6 yellow segments. The flower contains three yellow stamens that are 4.7 ± 1.4 cm long. Anthers are also yellow and 1.0 ± 1 cm long. Style (5.6 ± 1.9 cm long) is divided into 6 slender yellow branches at the top. Six branched stigmas are shown in Figure 8. Fruit of *C. olivieri* is an ellipsoid, oblong capsule. When it becomes mature, pedicel elongates and capsule is risen to just above the ground level. Seeds are oblong, blackish to dark brown and 1-3 mm long (Figure 9). The average weights of 1000 seeds were determined as 6,819 g.

Propagation by Corms

In order to achieve germination, *C. olivieri* corms, which were collected from the

sampling area, were stored at 4 ^{o}C and were planted in sand pots. After 50 ± 18 days, a shoot has emerged above the soil. About 58 days of planting, flowering took place and leaves appeared (Figure 10). Only 11 individuals out of 62 were flowered at indoor conditions. Others continued their development either without flowers or with only flower buds that were not open. Sprouts of 20 corms that were directly planted after collection from the field appeared on the sand at about 149 days. A new daughter corm was detected at the top of the previous corm that started to disintegrate and shrink (Figure 11).

In the field, however, corms started to sprout in October, and shoot arose on soil in March. It was also observed that there were contractile roots (Figure 12) between the mother corm and the daughter corm of the samples that were collected from the squeezed part of the soil on the side of the path. The diameter of the contractile roots was greater than that of the developing corm. About 3 months after flowering, the enlarged daughter corm maturated and it became dormant during summer.

Seed Viability

The biochemical (tetrazolium) and viable seed testing methods were applied for the seed viability. *C. olivieri* seeds showed 96 % viability at the tetrazolium test. The other viable seed testing showed that the germination percentage was 89 %. In this method, seeds were planted sand-perlite mixture. The seeds started to germinate after 78 days of planting and completed their germination after 161 days of planting. Thus, the germination period of the seeds were determined as 83 days.

Seed Germination

Among the different germination experiments. Two of them were found to be successful to achieve germination. One of these seeds was soaked in a water for about 24 hours. Then, the seeds were transferred into 1 cm deep of sand/perlite mixture and kept at room temperature by providing enough moisture. The other, seeds were soaked in water for 10 days. Then, they were transferred into a petridish containing moistened cotton layers, and were stored at room temperature by providing enough moisture. Seed germination requires 154-179 days after planting (Figure 13).

CONCLUSION

C. olivieri flowers in a 21-day period in spring between late March and late April. Approximately two months after the flowering, the seeds maturate. Then, the capsule opens and the seeds spread out to the soil in the beginning of July. This is followed by a dormancy period until October. In October, corm sprouting begins. Flowers and leaves appear in March. At the laboratory conditions, the flowers appeared in late December. The style of *C. olivieri* subsp. *olivieri* is divided into six branches at the top and its corm is membranous, splitting into parallel fibers at the base.

The color of flowers of the samples gathered in the study area is ranging from pale lemon to deep orange, as described by Mathew (1982). Weight of C. *olivieri* corm is continuously increasing while that of its leaves remains more or less unchanging during May, June, and July. When the leaves are about 40 cm long, they start to dry and disappear after June. Average weight of 1000 seeds was 6.819 grams. Corms germinated 50 ± 18 days after planting. However, during phenological observations, it is determined that 168 days are required only for sprouting. Indoor conditions and storing corms at 4 ^{o}C for a certain period hasten corm germination. Among the seed viability tests, Tetrazolium Test is better. It is more sensitive than Viable Seed Testing and the results could be obtained in very short period of time. *C. olivieri* seeds are not sterile. Keeping the seeds for 1-10 days in distilled water before planting, storing them in moist sand-perlite mixture or cotton layers at room temperature for about 154-179 days was ideal for seed germination. In this study, seed dormancy could not be broken.

As a conclusion, *C. olivieri* can be cultivated easily since its cultivation not only depends on corms and it produces none-sterile and numerous collectable seeds.

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Tables

Table 1. Lengths of phenological periods

	Sprouting period	Leaf appearing period	Flowering period	Fruit maturation period	Leaf disappearing period
Periods (days)	168	7	21	79	7

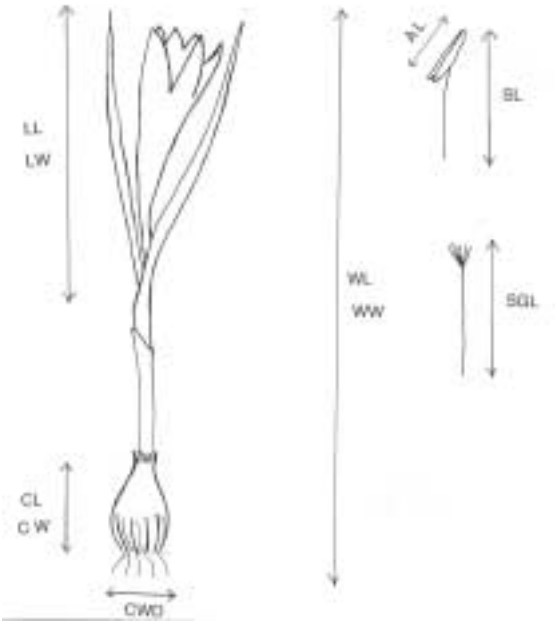
Figures

Fig. 1. *Crocus olivieri* Gay.

Fig. 2. *Crocus sativus* L.

Fig. 3. Labeling of individual plants

Fig. 4. Labeling of *C. olivieri* at the sampling area



LL:	Leaf Length	WL:	Whole Length
LW:	Leaf Weight	WW:	Whole Weight
CL:	Corm Length	SL:	Stamen Length
CW:	Corm Weight	SGL:	Stigma Length
CWD:	Corm Width	AL:	Anther Length

Fig. 5. Morphometric characters

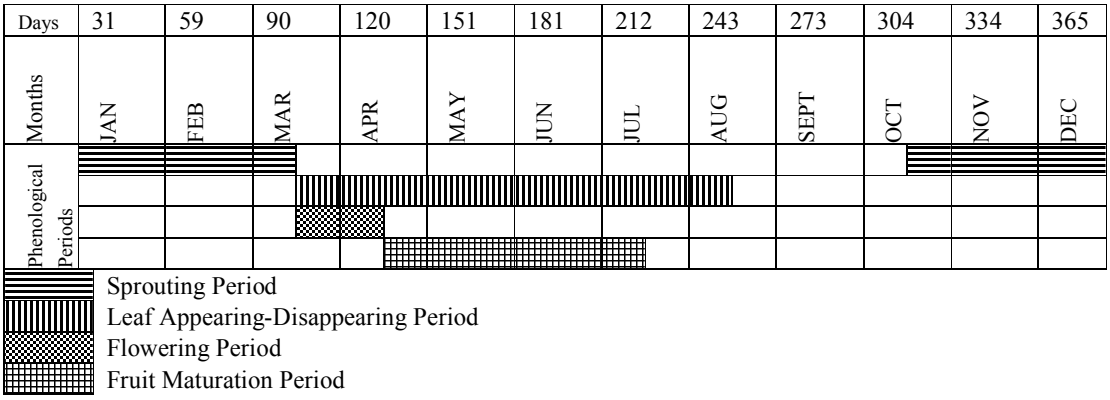


Fig. 6. Phenological periods according to months

Fig. 7. Corm tunic

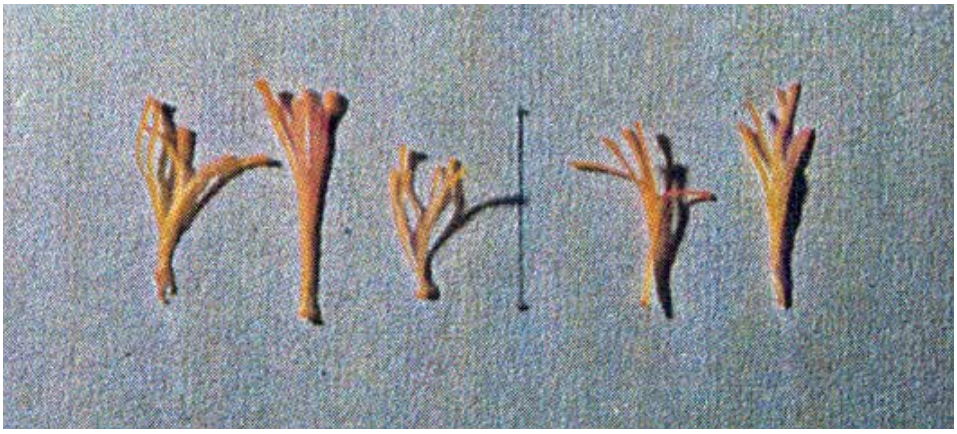


Fig. 8. Six branched *C. olivieri* stigmas

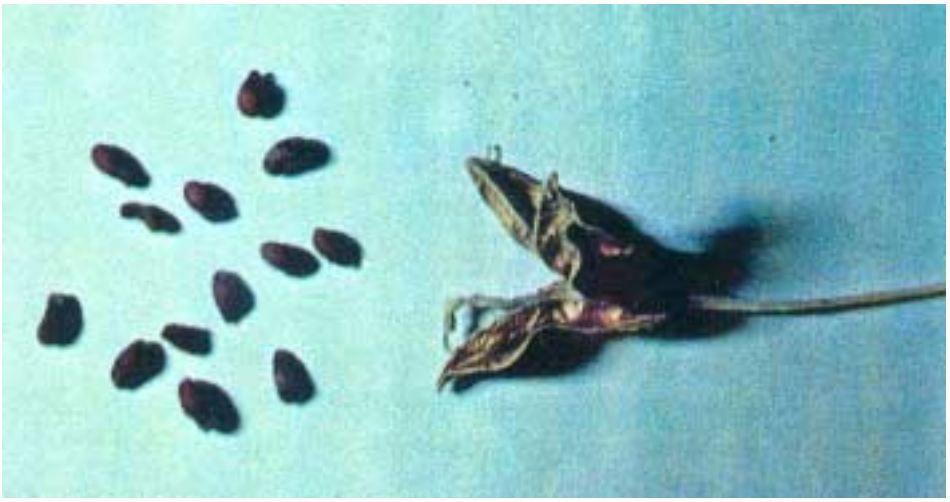


Fig. 9. *C. olivieri* capsule and seeds

Fig. 10. Flowering in sand pot at the laboratory

Fig. 11. Mother corm and daughter corm

Fig. 12. Contractile root

Fig. 13. Seed germination