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Seed Germination of Some Crocus Species of Western Anatolia[1]

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ABSTRACT *In this study, germination studies were carried out on some* ***Crocus*** *species such as* ***Crocus olivieri*** *ssp.* ***balansae***, ***Crocus chrysanthus***, ***Crocus baytopiorum***, ***Crocus pallasii*** *ssp.* ***pallasii*** *spreading at western Anatolia. Seed viability was determined by using tetrazolium test. Testa, endosperm, embryo bed of seeds were examined at stereo binocular. The pre-treatments for germination were; waiting seeds in moist sand at 5°C for 4 weeks, keeping seeds for 24 hours in water and in 200-400 mg/l GA_3 solutions; sowing directly to peat-filled viols. Seeds were sown between two blotting paper in petri dishes and kept at 10°C preserving enough humidity. The seeds of* ***Crocus baytopiorum*** *were germinated 25 days after sowing,* ***Crocus olivieri*** *ssp.* ***balansae*** *seeds were started to germinate within 33 days and the seeds of* ***Crocus chrysanthus*** *germinated in 37 days. For the treatment of sowing seeds directly to peat filled pots, the germination started 2 months later. The rate of emergence for* ***Crocus olivieri*** *ssp.* ***balansae*** *was 14 %;* ***Crocus chrysanthus*** *was 82.5 %;* ***Crocus baytopiorum*** *was 73.8 % and* ***Crocus pallasii*** *ssp.* ***pallasii*** *was 73.5 %. At* ***in vitro*** *conditions seeds were sown to MS medium without plant growth regulators with pre-treatment of waiting seeds for 24 hours in solutions of 250 and 500 mg/L GA_3. The germination rate was higher at treatment of 500 mg/L GA_3 than 200 mg/L GA_3.* ***In vitro*** *seed germination was started 8 months later and the rates were 25 % at* ***Crocus olivieri*** *ssp.* ***balansae****; 9.5 % at* ***Crocus chrysanthus****; 80 % at* ***Crocus baytopiorum*** *and 90 % at* ***Crocus pallasii*** *ssp****. pallasii****.*

Key words: Crocus *sp., seed, germination*

Batı Anadolu'daki Bazı Crocus Türlerinde Tohum Çimlenmesi

ÖZ: *Bu çalışmada, Batı Anadolu'daki* ***Crocus olivieri*** *ssp.* ***balansae***, ***Crocus chrysanthus***, ***Crocus baytopiorum***, ***Crocus pallasii*** *ssp.* ***pallasii*** *gibi bazı doğal* ***Crocus*** *taksonlarının tohum çimlendirme çalışmaları yürütülmüştür. Tohum canlılığı tetrazolium testi ile belirlenmiştir. Tohumların tohum kabuğu, endosperm ve embriyo gibi kısımları stero binokülerde incelenmiştir. Tohumları 5 °C'de 4 hafta nemli kumda bekletme, suda ve 200-400 mg/l'lik GA_3 çözeltilerinde 24 saat tutma; torf doldurulmuş viyollere doğrudan ekme çimlenme çalışmalarının ön uygulamalarını oluşturmuştur. Tohumlar, petri kapları içine yerleştirilmiş nemli 2 adet kurutma kâğıdının arasına ekilmiş ve yeterli nemi korunarak 10 °C'de tutulmuştur.* ***Crocus baytopiorum*** *tohumları ekimden 25 gün sonra çimlenmiştir.* ***Crocus olivieri*** *ssp.* ***balansae*** *tohumları çimlenmeye 33 gün içinde başlamıştır.* ***Crocus chrysanthus*** *tohumları 37 günde çimlenmiştir. Tohumları doğrudan viyollere ekme uygulamasında çıkışlar ekimden 2 ay sonra olmuştur. Çıkış oranları* ***Crocus olivieri*** *ssp.* ***balansae****'de %14;* ***Crocus chrysanthus****'ta % 82,5;* ***Crocus baytopiorum****'da % 73,8 ve* ***Crocus pallasii*** *ssp.* ***pallasii****'de % 73,5 olarak belirlenmiştir.* ***In vitro*** *koşullarda tohumlar bitki büyümeyi düzenleyici içermeyen MS ortamına tohumların 24 saat 250 ve 500 mg/L GA_3 çözeltilerinde bekletildiği ön uygulamalardan sonra ekilmiştir. Çimlenme oranı 500 mg/L GA_3 ön uygulamasında 250 mg/L GA_3 ön uygulamasından daha yüksek bulunmuştur.* ***In vitro*** *tohum çimlenmesi ekimden 8 ay sonra başlamış ve oranlar* ***Crocus olivieri*** *ssp.* ***balansae****'de % 25;* ***Crocus chrysanthus****'ta %9,5;* ***Crocus baytopiorum****'da % 80 ve* ***Crocus pallasii*** *ssp****. pallasii****'de % 90 olarak belirlenmiştir.*

Anahtar sözcükler: Crocus *sp., tohum, çimlenme*

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INTRODUCTION

Genus of *Crocus* is horticulturally important as autumn, winter and spring flowering corms (Benschop, 1993). The colors of flowers differ to white, yellow, blue and purple. The leaves are seen with flowers or after flowers and continue to develop after flowering (Vurdu and Cicek, 1992).

In the world there are approximately 80 *Crocus* species which all of them are seen at northern hemisphere. Most of the identified species are spreading at Balkans and Turkey (Erol *et al*., 2011; Şık *et al*., 2008; Vurdu *et al*., 2004). *Crocus* genus has 72 taxa in Turkey. The second genus is *Iris*, both of them are belong to the family of *Iridaceae*. *Crocus* flowers have beautiful colors and they are unique because of blossoming when there are not so many flowers at environment. They are flowering before tulips in spring time and they can plant with tulips and narcissus in flower bulb gardens.

In this study it is aimed to cultivate *Crocus* taxa which are spreading naturally at Western Anatolia in Turkey. Seed germination of *Crocus* species is one of the steps of cultivation of these plants.

MATERIALS AND METHODS

The materials of the study are 4 *Crocus* taxa which spreading naturally at flora of Turkey and including 2 endemics. While one of them blossoms during autumn (*C. pallasii* Goldb. subsp. *pallasii*), the other three (*C. olivieri* J. Gay ssp. *balansae* (Ga ex Baker) B. Mathew, *C. chrysanthus* Herb., and *C. baytopiorum* B. Mathew) blossom during spring.

In our study, we picked up the taxa around the regions where they spread in Western Anatolia and their species were identified.

Seed germination studies were carried out in 2009 and 2010. Seeds of 3 taxa, blossoms in spring, were used for germination studies with pre-treatments in 2009. In 2010 with the addition of 1 autumn blossoming taxon as material, seeds of 4 *Crocus* taxa were sown at field conditions.

The studies were carried out Randomized Design with 4 replicates and each replicate had 25 seeds.

Viability tests

TTC test is used to examine seed viability. The testa of seeds were holed and waited in water at 30 ^{o}C for 20 hours. Later the seeds were kept in 1 % 2, 3, 5-triphenyltetra-zolium chloride (TTC) solution for 24 hours at 30 ^{o}C. Seeds were cut horizantaly and the seeds with stained embriyos were considered as viable (Kose, 1997).

Morphological Assessments

For the morphological assessments, seed width and length were measured using 30 seeds, weight of 100 seeds were determined. We used "Methuen Handbook of Colour" for detecting color (Kornerup and Wanscher, 1978). After TTC test, the stained seeds were horizantaly cut to thin layers; testa, embryo bed and endosperm of seeds were examined at stereo binocular.

Pre-treatments (PT)

PT1: The seeds were kept for 24 hours in distilled water and were sown into vials which were filled with turf and placed at land conditions (Cicek, 1994).

PT2: Waiting seeds in moist sand at 4^{o}C for 4 weeks, than seeds were sown between two blotting paper in petri dishes and kept at 10^{o}C preserving enough humidity.

PT3: Keeping seeds for 24 hours in water and in 200 mg/l GA_3(Gibberellic acid) solution and seeds were sown between two blooting paper in petri dishes and kept at 10^{o}C preserving enough humidity (Cicek, 1994).

PT4: Keeping seeds for 24 hours in water and in 400 mg/l GA_3 solution and seeds were sown between two blotting paper in petri dishes and kept at 10^{o}C preserving enough humidity (Cicek, 1994).

Control Group: Seeds sown without pre-treatment between two blooting paper in petri dishes and kept at 10^{o}C preserving enough humidity.

Direct germination: Sowing seeds into peat-filled viols at land conditions.

***In vitro* seed germination**

In vitro seed germination studies were conducted in 2010. The seeds were kept at 250 and 500 mg /l GA_3 solutions for 24 hours and sown into MS Murashige and Skoog (MS) medium without plant growth regulators.

RESULTS

Seed widht of *Crocus olivieri* ssp. *balansae* differed between 1.5 mm and 2.0 mm; seed lenght were measured between 3.4 mm-4.2 mm. For *Crocus chrysanthus* seed width were measured 1.6 mm to 2.2 mm and seed length were 3.0 mm – 4.7 mm. For *Crocus baytopiorum* the measurement of seed width were between 1.6 mm and 2.1 mm; seed length differed between 2.6 mm – 4.2 mm. For *C. pallasii* ssp. *pallasii* seed width values were differed between 2.1 and 3.2, seed length were 3.3 and 4.8. Average value can be seen at Table 1 for 2009 and 2010. Testa, embryo bed and endosperm of seeds can be seen at Figure 1 and for each taxa at Figure 2.

With the pre-treatments of seeds germination the best results were taken from the PT-1 (waiting seed 24 hours in distiled water and sowing into turf filled viols) (Table 2). But germination was stopped 4 months later in 2009. After that in 2010 seeds were sown directly into turf filled viols without pre-treatment. In this treatment germination started in 2 months after sowing. Germination rates were 14.0 % for *Crocus olivieri* ssp. *balansae*; 82.5 % for *Crocus chrysanthus*; 73.8 % for *Crocus baytopiorum* and 73.5 % for *Crocus pallasii* ssp. *pallasii*.

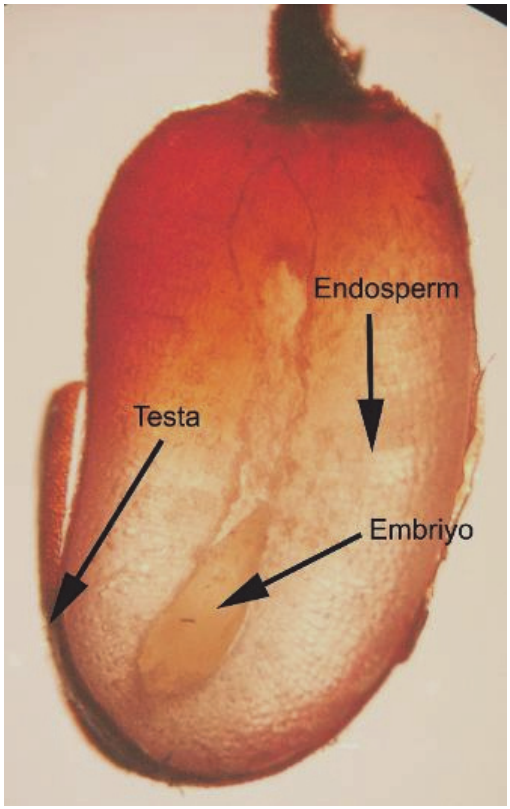

Fig. 1. Testa, embryo bed and endosperm of *Crocus* seeds
Şekil 1. *Crocus* tohumlarının tohum kabuğu, embriyo ve endosperm kısımları

Table 1. Morphological assesments of *Crocus* taxa for the years 2009 and 2010
Çizelge 1. *Crocus* taksonlarının 2009 ve 2010 yılı için morfolojik özellikleri

Taxa Taksonlar	Average seed width Ortalama tohum eni (mm)		Average seed length Ortalama tohum boyu (mm)		100 Seeds weight 100 tohum ağıtlığı (g)		Seed color Tohum rengi
	2009	2010	2009	2010	2009	2010	
C. olivieri ssp. *balansae*	1.8	1.7	3.4	3.5	0.64	0.57	13/5F
C. chrysanthus	1.8	1.8	3.8	4.3	0.76	0.70	9/6D
C. baytopiorum	1.8	2.1	3.3	4.0	0.42	0.69	9/6F
C. pallasii ssp. *pallasii*	2.7	2.8	3.8	3.8	1.67	1.64	8/8F

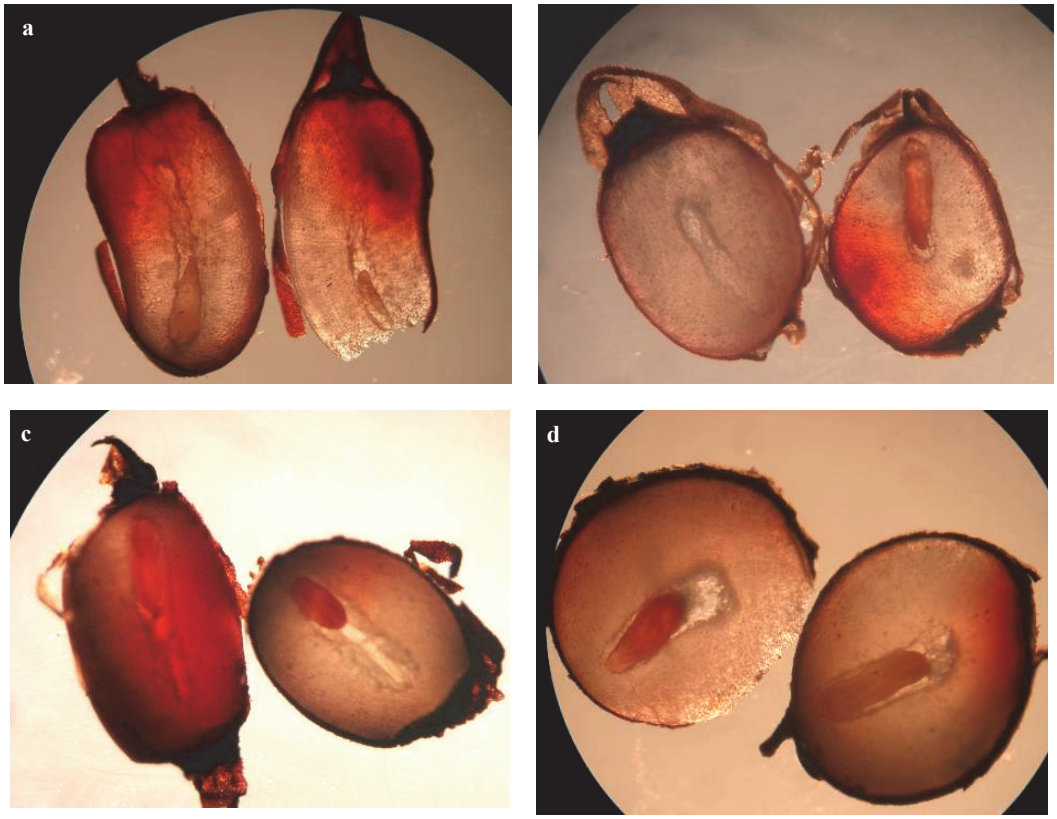

Fig. 2. Horizontal section of stained Seeds a) *Crocus olivieri* ssp. *balansae*, b) *Crocus chrysanthus*, c) *Crocus baytopiorum*, d) *Crocus pallasii* ssp. *pallasii.*
Şekil 2. Boyanmış tohumların enine kesitleri; a) *Crocus olivieri* ssp. *balansae*, b) *Crocus chrysanthus*, c) *Crocus baytopiorum*, d) *Crocus pallasii* ssp. *pallasii.*

Table 2. Germination rates of seeds after pre-treatments
Çizelge 2. Ön uygulamalar sorması tohumların çimlenme yüzdeleri

	C. olivieri ssp. *balansae*	*C. chrysanthus*	*C.baytopiorum*
PT 1	58.0	70.0	100.0
PT 2	5.0	2.0	50.0
PT 3	18.0	2.0	62.5
PT 4	10.0	4.0	62.5
Control (Kontrol)	11.0	1.0	50.0

In vitro germination started within 10 months and cormlets developed in 12 months. For *Crocus olivieri* ssp.*balansae*, germination rate was 25 % and cormlet rate was 6 % with pre-treatment of 500 mg /L GA_3; for 250 mg /L GA_3 pre-treatment germination rate was 12 % and no cormlet existed. For *Crocus chrysanthus* germination rate was high (14.3 %) without pre-treatment but there was no cormlet. With pre-treatment of 500 mg /L GA_3 germination rate was 9.5 % and cormlet rate was 4.8 %. For *Crocus baytopiorum* the highest germination (80 %) and cormlet (40 %) rates were obtained by the pre-treatment of 500 mg /L GA_3. For *Crocus pallasii* ssp. *pallasii* pre-treatment with 500 mg /L GA_3 took the same value, both germination and cormlet rate was 90 % (Table 3).

Table 3. *In vitro* seed germination and cormlet rate
Çizelge 3. *In vitro* tohum çimlenmesi ve kormlet oranı

Taxa Taksonlar	Pre-treatment Ön uygulama	Germination rate Çimlenme oranı(%)	Cormlet rate Kormlet oranı (%)
C. olivieri ssp. *balansae*	-	0.0	0.0
	250 mg/l GA_3	12.0	0.0
	500 mg/l GA_3	25.0	6.0
C. chrysanthus	-	14.3	0.0
	250 mg/l GA_3	0.0	0.0
	500 mg/l GA_3	9.5	4.8
C. baytopiorum	-	28.6	0.0
	250 mg/l GA_3	4.4	6.7
	500 mg/l GA_3	80.0	40.0
C. pallasii ssp. *pallasii*	-	0.0	0.0
	250 mg/l GA_3	23.7	5.3
	500 mg/l GA_3	90.0	90.0

DISCUSSION

The seeds of taxa were reacted differently to the pre-treatments for seed germination. The seeds which were sown directly without pre-treatment had high germination percentage in 2 months. For *in vitro* conditions the seeds germinated and we get cormlet but the percentage was not high. This results shows that the seeds of natural *Crocus* plants need no special conditions for germination. The researchers who are studying genus of *Crocus* indicated that they had similar results (N. Arslan, 2009, personal communication).

Çiçek (1994), was carried out seed germination studies and used the seeds of *Crocus oliveri* J. Gay subps. *oliveri* with 10 different pre-treatment. The most important results were; only 2 out of 10 pre-treated seeds which were soaked 1 and 10 days in distilled water were germinated. The pre-treatment which seeds were waited 1 day and sown into sand and perlite mixture, germination was started after 154 days and germination percentage was 89 %. The same pre-treatment was used in this study and similar results were detected. For *Crocus oliveri* ssp. *oliveri* germination rate was 58 %, this result is lower than results of *Crocus oliveri* J. Gay subps. *oliveri.* For *Crocus chrysanthus* germination rate was 70 % and lower than *Crocus oliveri* J. Gay subps. *oliveri.* The best results were observed in *Crocus baytopiorum* with 100 % germination rate.

Kravkaz (2008) carried out seed germination studies with the seeds of *Crocus ancryrensis* Maw ve *C. speciosus* M.Bieb. subsp. *ilgazensis* B.Mathew. The pre-treatments of study were; firstly hot (20 ^oC) stratification for 4 weeks and later cold (4 ^oC) stratification for 4 weeks. After stratification treatments were waiting seeds in water for 24 hours and wating for 4 hours in 250-500-750 ppm GA_3 solutions. Then the seeds with pre-treatments were kept at 20 ^oC in climate fridge at first year and at 10 ^oC in climate fridge at second year. 10 ^oC and 70 % humidity was more suitable than 20 ^oC with 90 % humidity. At 10 ^oC without stratification germination of seeds started in 2 months. After stratification treatments, seed germination started 1 month later, but stratification studies also needs 2 more months, as a result it needs totally 3 months. In stratification treatments germination percentage (77-88 %) was higher than without stratification (65-66 %). Waiting seeds for 4 hours in 250 ppm GA_3 solution was the most effective treatment for germination (80 %). In this study PT-1(waiting distilled water for 24 hours and sowing into vials) was the best pre-treatment. With pre-treatments the seeds were kept at 10 ^oC aproximately 2 years and the germination started 25-37 days after sowing. However, germination was not completed for seeds of both *Crocus oliveri* subps. *oliveri* and *Crocus chrysanthus*. Erken (2009), stated that the seed of *Iris* genus also needs

more than 2 years for germination at controlled conditions.

In this study seeds were sown directly into turf filled vials and germination was started after 2 months. It is indicated that germination and corm formation of these seeds do not need special conditions, they can germinate at field conditions easily when they are sown in october. However, considering negative impact of environment for field conditions, future studies should be needed on controlled conditions to determine full procedures for germination of *Crocus* genus seeds.

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