Bangladesh J. Bot. **46**(3): 939-946, 2017 (September)

SEED GERMINATION RESPONSES OF SOME *VERBASCUM* L. SPECIES TO DIFFERENT COLD-WET PRE-TREATMENTS AND PHOTOPERIOD PROCESSES

GULCIN ISIK*, FAIK A KARAVELIOGULLARI[1], ERSIN YUCEL AND SEZGIN CELIK[2]

Department of Biology, Faculty of Sciences, Anadolu University, 26470, Eskişehir, Turkey

Keywords: Germination, Photoperiod, Seed, *Verbascum*

Abstract

Seed germination responses of six *Verbascum* L. species to different cold-wet pre-treatments and photoperiod processes were examined. *V. dudleyanum, V. natolicum, V. serratifolium* and *V. suworowianum* var. *suworowianum* had the highest germination percentage at 22ºC fixed temperature, 8 h light/16 h dark photoperiod application after ten-day long cold-wet pre-treatment. *V. orientale* had the highest germination percentage at 8 hrs light/16 hrs dark photoperiod application after 48 hrs long cold-wet pre-treatment. *V. wiedemannianum* had the highest germination percentage at 16 hrs light/8 hrs dark photoperiod application after ten-day long cold-wet pre-treatment.

Introduction

The genus *Verbascum* L. (*Scrophulariaceae*) includes about 360 species (Heywood 1993). In Turkey, it is represented by 233 species in 13 groups, and 126 hybrids (Huber-Morath 1978), 187 (80%) of these 233 species are endemic (Davis *et al.* 1988). In Flora of Europe (Ferguson 1972) this genus is represented by 99 species, in Flora of the U.S.S.R (Fedchenko 1955) by 51, in Flora Iranica (Huber-Morath 1981) by 49, in Flora Palestine (Feinbrun-Dothan 1978) by 20, in Flora of Cyprus (Meikle 1985) by six and in Flora of Egypt (Tackholm 1974) by four. The diversity is highest in Anatolia. The plants are adapted especially to steppe environment, open places and stony slopes. *V. dudleyanum* is a perennial, 30 - 80 cm long, glabrous and herbaceous plant. Flowers are yellow and often red colored spotted at center. This species is endemic for Turkey, an Irano-Turan element. *V. natolicum* is a biennial, 50 - 100 cm long and herbaceous plant. Flowers are yellow. This species is endemic for Turkey, an Irano-Turan element and generally spreads in Central Anatolia. *V. orientale* is an annual, 15 - 80 cm long, sparsely hairy and herbaceous plant. Flowers are yellow and brown colored spotted rarely. This species is an East Mediterranean element and toxic. *V. serratifolium* is a perennial, 50 - 120 cm long and herbaceous plant. Flowers are yellow and glabrous at outer side. This species is endemic for Turkey, an Irano-Turan element. It is valuable as an ornamental plant. *V. suworowianum* var. *suworowianum* is a biennial or perennial, 25 - 70 cm long and herbaceous plant. Flowers are yellow, glabrous or sparsely glandular hairy at outer side; stem is glandular hairy. This taxon is an Irano-Turan element. *V. wiedemannianum* is a biennial, 35-125 cm long and herbaceous plant. Flowers are violet. This species is endemic for Turkey, an Irano-Turan element. It includes chemical compounds such as iridoids, triterpen saponin and phenylethanoid glycosides (Abou *et al.* 2003, Calis *et al.* 2002). It is known that seeds of some *Verbascum* species can survive more than 100 years (Forey *et al.* 2011).

*****Author for correspondence: <glcnylmz@gmail.com>. [1]Hakkari University, Yüksekova Vocational High School, Hakkari, Turkey. [2]Department of Molecular Biology and Genetics, Faculty of Sciences, Yıldız Technical University, Beşiktaş/İstanbul, Turkey.

The increase of climatic variability due to global climate change exerts climatic stress on plants that is novel in magnitude and frequency (Walter *et al.* 2013). Among the germination factors (Ghaderi *et al.* 2008), temperature is the most prominent environmental factor regulating plant growth and development (Koger *et al.* 2004). The optimum temperature at which the maximum germination and emergence percentage are recorded tends to differ among crops (Motsa *et al.* 2015). The requirement of light for the germination of seeds of certain plant species prevents germination in places and times not favourable for seedling establishment (Fenner and Thompson 2005, Motsa *et al.* 2015). The light requirement of such seed acts as a mechanism that determines where and when germination takes place, and is important for survival of the plant species concerned, as it prevents stored seed reserves from being depleted (Motsa *et al.* 2015). Some seeds germinate equally well in light and darkness, whilst others germinate better under only light or darkness (Chanyenga *et al.* 2012).

The aim of this study was to measure the effect of different photoperiod and cold-wet pre-treatment applications on seed germination characteristics of some *Verbascum* L. species (*V. dudleyanum*, *V. natolicum*, *V. orientale*, *V. serratifolium*, *V. suworowianum* var. *suworowianum* and *V. wiedemannianum*) and to demonstrate the differences between these species.

Materials and Methods

After determining the natural distribution area of plants, the plant samples were collected from six different natural areas of Turkey (Table 1). The plant samples collected are deposited in the herbarium of Faculty of Sciences and Arts, Gazi University. The seeds were set apart from the plant samples at room temperature and after cleaning. The germination experiments were carried out in plant growth chambers (MLR-350 Model Sanyo, Japan). Germination tests were performed in Petri dishes (9 cm diam) with two discs of filter papers and one hundred seeds were set at each Petri dish. Germination experiments were repeated four times (4 × 100). The seeds used for experiments were arranged into three groups. For the first group, there was no pre-treatment application; the second one had 48 hrs long (4ºC) cold-wet process; the 31 had ten-day long (4ºC) cold-wet process. After pretreatment, the seeds were transferred into germination experiments at fixed temperature (22ºC); continuous dark, 8 hrs light/16 hrs dark and 16 hrs light/8 hrs dark photoperiods. In order to consider the seed to be germinated, the radicle must touch the germination bed. During the experiments, checkings were done at a definite time everyday and the seeds that germinated were removed from dishes after enumerating. The quantity obtained was recorded in the experimental table. For evaluation of the results obtained from the germination experiments, the "ANOVA F Test" was applied.

Results and Discussion

Verbascum dudleyanum had the highest germination percentage (30) at 22ºC fixed temperature, 8 hrs light/16 hrs dark photoperiod application after ten day long cold-wet process (Table 2). It had the lowest germination percentage (2) at continuous dark application and there was no process. The results were statistically meaningful with 95% reliability. *V. natolicum* had the highest germination percentage (65) at 22ºC fixed temperature, 8 hrs light/16 hrs dark photoperiod application after ten-day long cold-wet process. It had the lowest germination percentage (13) at continuous dark application and there was no process. *V. orientale* had the highest germination percentage (98) at 22ºC fixed temperature, 8 hrs light/16 hrs dark photoperiod application after 48 hrs long cold-wet process. It had the lowest germination percentage (14) at continuous dark application after ten-day long cold-wet process. *V. serratifolium* had the highest germination percentage (47) at 22ºC fixed temperature, 8 hrs light/16 hrs dark photoperiod

application after ten-day long cold-wet process. It had the lowest germination percentage (1) at unprocessed 8 hrs light/16 hrs dark photoperiod application. *V. suworowianum* var. *suworowianum* had the highest germination percentage (90) at 22ºC fixed temperature, 8 hrs light/16 hrs dark photoperiod application after ten day long cold-wet process. It had the lowest germination percentage (27) at unprocessed and 16 hrs light/8 hrs dark photoperiod application. *V. wiedemannianum* had the highest germination percentage (87) at 22ºC fixed temperature, 16 hrs light/8 hrs dark photoperiod application after ten-day long cold-wet process. It had the lowest germination percentage (8) at unprocessed and 8 hrs light/16 hrs dark photoperiod application.

Table 1. Seed collection localities.

Botanical name	Locality	Latitude-longitude	Herbarium No. (GAZI)
V. dudleyanum	Burdur, Salda Lake, 850-900 m	N 37°29'08.4'' E 28°36'45.2''	FAK 3323
V. natolicum	Erzurum, Olur, Coşkunlar Village, 1045 m	N 40°45'38.3'' E 42°10'44.6''	FAK 3008
V. orientale	Erzurum, Olur, Yeşilbağlar Village, Parsidan Stream, 1074 m	N 40° 47'38.7'' E 42°08'55.0''	FAK 3359
V. serratifolium	Eskişehir, Porsuk Dam, 902 m	N 39°44'48.0'' E 30°26'37.6''	FAK 3368
V. suworowianum var. *suworowianum*	Kars, Kağızman, Horasan Road, 14-16. km, 1281 m	N 40°06'01.9'' E 42°58'48.1''	FAK 3362
V. wiedemannianum	Sivas, Taşlıdere, 1360 m	N37°36'16.4'' E 37°01'75.2''	FAK 3324

The cold-wet pre-treatment which was applied to the seeds was important in point of statistics at the level of $p \leq 0{,}001$ (Table 3) and it was found that there were different independent groups formed for each process (Table 4).

It was found that different photoperiods which were applied to the seeds during germination were impressive on germination and this effect was significant at $p \leq 0{,}001$ level (Table 5). When the results of germination experiments were compared it was observed that there was a difference between species and formed five groups which are different from each other (Table 6).

According to that, ten days long cold-wet pre-treatment and 8 hrs light/16 hrs dark photoperiod application promoted the development of germination at *V. dudleyanum*, *V. natolicum*, *V. serratifolium*, *V. suworowianum* var. *suworowianum* and *V. wiedemannianum.* On the other hand, long periodic cold-wet pre-treatment handicapped germination at *V. orientale.*

It was observed that the species divided to two main groups from the cluster analysis of data obtained from seed germination experiment. While the members of first group are *V. dudleyanum, V. natolicum* and *V. orientale*, but this species was separated from two of other species in this group significantly. Second group consists of *V. serratifolium*, *V. suworowiaunum* var. *suworowiaunum* and *V. wiedemannianum.* The behavior of seed germination of these groups is similar (Fig. 1).

Table 2. Seed germination characteristics of some *Verbascum* species at different process and photoperiod applications.

Botanical name	Process	Application (22°C)	Germination (%)
V. dudleyanum	No pre-treatment	8 h L/16 h D	11 c
		16 h L/8 h D	6 a
		Dark	2 a*
	48 hrs cold-wet pre-treatment	8 h L/16 h D	17 d
		16 h L/8 h D	11 c
		Dark	5 b
	10 days cold-wet pre-treatment	8 h L/16 h D	29 e
		16 h L/8 h D	19 d
		Dark	9 c
V. natolicum	No pre-treatment	8 h L/16 h D	20 b
		16 h L/8 h D	15 a
		Dark	13 a*
	48 hrs cold-wet pre-treatment	8 h L/16 h D	41 d
		16 h L/8 h D	26 b
		Dark	15 a
	10 days cold-wet pre-treatment	8 h L/16 h D	65 f
		16 h L/8 h D	59 e
		Dark	31 c
V. orientale	No pre-treatment	8 h L/16 h D	97 c
		16 h L/8 h D	96 c
		Dark	93 c
	48 hrs cold-wet pre-treatment	8 h L/16 h D	98 c
		16 h L/8 h D	96 c
		Dark	95 c
	10 days cold-wet pre-treatment	8 h L/16 h D	23 b
		16 h L/8 h D	25 b
		Dark	14 a*
V. serratifolium	No pre-treatment	8 h L/16 h D	1 a*
		16 h L/8 h D	0 -
		Dark	0 -
	48 hrs cold-wet pre-treatment	8 h L/16 h D	1 a
		16 h L/8 h D	1 a
		Dark	1 a
	10 days cold-wet pre-treatment	8 h L/16 h D	47 d
		16 h L/8 h D	29 b
		Dark	33 c
V. suworowianum var. *suworowianum*	No pre-treatment	8 h L/16 h D	53 b
		16 h L/8 h D	27 a*
		Dark	34 a
	48 hrs cold-wet pre-treatment	8 h L/16 h D	89 e
		16 h L/8 h D	71 d
		Dark	62 c
	10 days cold-wet pre-treatment	8 h L/16 h D	90 e
		16 h L/8 h D	76 d
		Dark	89 e
V. wiedemannianum	No pre-treatment	8 h L/16 h D	8 a*
		16 h L/8 h D	43 d
		Dark	12 a
	48 hours cold-wet pre-treatment	8 h L/16 h D	25 b
		16 h L/8 h D	62 e
		Dark	40 c
	10 days cold-wet pre-treatment	8 h L/16 h D	62 e
		16 h L/8 h D	87 f
		Dark	84 f

L: Light, D: Dark, h: Hour; *: Within each column, means with the same letter are not significantly different (p = 0.05)

Table 3. Tests of between-subjects effects (dependent variable: germination).

Source	Type III sum of squares	Df	Mean square	F	Sig.
Corrected model	236286,259	29	8147,802	95,132	0.000
Intercept	342885,352	1	342885,352	4003,460	0.000
Species	129008,370	5	25801,674	301,255	0.000
Process	15664,843	2	7832,421	91,450	0.000
Photoperiod	4326,009	2	2163,005	25,255	0.000
Species process	73758,935	10	7375,894	86,119	0.000
Species photoperiod	13528,102	10	1352,810	15,795	0.000

a R squared = 0.937 (Adjusted R squared = 0.927).

Table 4. Germination, DMRT.

	N	Subset		
Process		1	2	3
1	72	28,76		
2	72		41,29	
3	72			49,47
Sig.		1,000	1,000	1,000

Table 5. Germination, DMRT.

	N	Subset	
Photoperiod		1	2
3	72	33,51	
1	72		42.96
2	72		43.06
Sig.		1,000	0.950

Table 6. Germination, DMRT.

	N	Subset				
Species		1	2	3	4	5
1	36	10,92				
4	36	12,31				
2	36		29,47			
6	36			46,69		
5	36				65,64	
3	36					74,03
Sig.		0.525	1,000	1,000	1,000	1,000

For *V. dudleyanum*, *V. natolicum* and *V. serratifolium,* comparing the results on point of pre-treatment applications showed that cold-wet pre-treatment promoted the germination, the highest germination rate is at 10 days long cold-wet pre-treatment, difference is significant at $p \leq 0.05$ level. Although 8 hrs light/16 hrs dark photoperiod promoted the seeds of this species for

germination, it was found out that ten days long cold-wet pre-treatment was the actual promotion factor on seed germination.

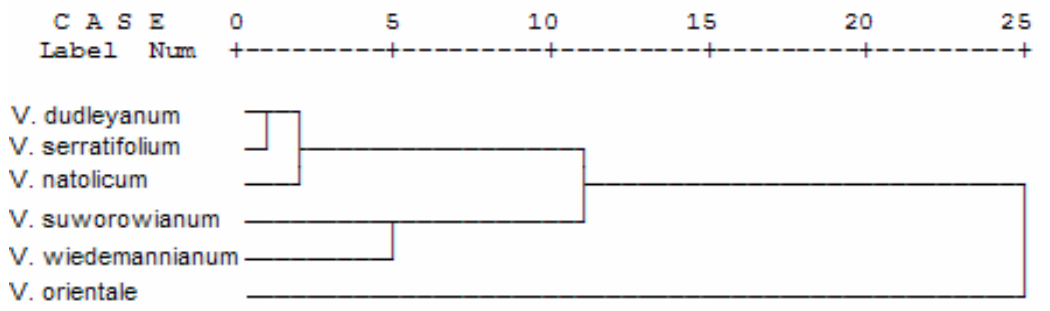

Fig. 1. Hierarchical cluster analysis, dendrogram using average linkage (intergroups), re-scaled distance cluster combine

Comparing the results for *V. orientale* and *V. suworowianum* var. *suworowianum,* 0n point of pre-treatment applications, presents that cold-wet pre-treatment promoted the germination, the highest germination rate was at 48 hrs long cold-wet pre-treatment, difference was significant at $p \leq 0,05$ level. According to these data, *V. orientale* and *V. suworowianum* var. *suworowianum* had the most appropriate germination medium at 22ºC fixed temperature, 8 hrs light/16 hrs dark photoperiod application after 48 hrs long cold-wet pre-treatment. It was found out that ten days long cold-wet pre-treatment was handicapped the germination for these taxa.

According to our data, *V. wiedemannianum* had the most appropriate germination medium at 22ºC fixed temperature, 16 hrs light/8 hrs dark photoperiod application after ten days long cold-wet pre-treatment. Although 16 hrs light/8 hrs dark photoperiod promoted the seeds of this species for germination, it was found out that ten days long cold-wet pre-treatment was the actual promotion factor on seed germination.

In the light of statistical analyses, six different groups were observed. First group, which is symbolized with "a" (Table 2), is consisted of no or short pretreatments. Group symbolized with "b" refers to dark treatments, "c" means experiment results of *V. orientale* which were very similar, "d" stood for 48 hrs or 10 days cold-wet treatments, "e" signified 10 days cold-wet treatments and "f" is *V. wiedemannianum*'s 10 days applications.

Although Sanchez-Romero *et al.* (2007) reported that cold-wet pre-treatment was not efficient on seed germination, after the experiments we had done, importance of long cold-wet pre-treatment on seed germination was found out, especially for seeds of *V. serratifolium*. The natural distribution area of this taxon, which is an Irano-Turanian element, is Erzurum and average annual temperature of this city is low (Anon. 2007). In this study, apart from *V. orientale,* which is a East Mediterranean element, it was observed that cold-wet pre-treatment is stimulator on seed germination. *V. orientale*'s ancient populations were adapted to Mediterranean climate which is temperate. However, other *Verbascum* species which are mentioned in this study belonged to Irano-Turanian or Euro-Siberian elements, we can assume that these species are adapted to non-temperate climate much more than East Mediterranean elements. We can say that the biogeographical regions of plant samples and their cold period required before and during germination is related. It was known that light is efficient on seed germination (Çırak *et al.* 2007, Kulkarni *et al.* 2007, Martinez-Sanchez *et al.* 2006), in this study we observed the significant

importance of light on seed germination also. Endemic species could be very delicate to ecological changes, so their seeds and germination behavior were more important both for storage and spreading seeds.

References

Abou H, Taşdemir D, Ireland CM and Çalış I 2003. Iridoids and triterpene saponins from *Verbascum wiedemannianum* (Scrophulariaceae). Bioch. Syst. and Eco. **31**(4): 433-436.

Anonymous. 2007. "http://www.meteor.gov.tr/2006/tahmin/tahmin-iller-erzurum.aspx?secimNo=31".

Calis I, Abou Gazar H, Bedir E and Khan I A 2002. Phenylethanoid glycosides with free radical scavenging properties from *Verbascum wiedemannianum*. Biodiversity. pp. 257-260.

Chanyenga TF, Gelldenhuys C J, Sileshi G W 2012. Germination response and viability of an endangered tropical conifer *Widdringtonia whytei* seeds to temperature and light. South Afr. J. Bot. **81**: 25-28.

Çırak C, Kevseroğlu K and Ayan AK 2007. Breaking of seed dormancy in a Turkish endemic *Hypericum* species: *Hypericum aviculariifolium* subsp. *depilatum* var. *depilatum* by light and some pre-soaking treatments. J. Ar. Envir. **68**: 159-164.

Davis PH, Mill RR and Tan K 1988. *Verbascum* L. *In:* Davis, P.H., Mill, R.R. & Tan, K. (eds.), Flora of Turkey and the East Aegean Islands (suppl.) 10: 191-193, Edinburgh Univ. Press, Edinburgh.

Fedchenko BA 1955. *Verbascum* L. *In:* Schischkin, B.K., Bobrow, E.G, Flora of USSR. 22: 132-197, Izdatel'stvo Akademii Nauk SSSR Moscow-Leningrad.

Feinbrun-Dothan N 1978. *Verbascum* L. *In:* Zohary, M., & Feinbrun-Dothan, N., Flora Palaestina Tex 3: 282-302, Jerusalem.

Fenner M and Thompson K 2005. The Ecology of Seeds. Cambridge University Press, Cambridge.

Ferguson I K 1972. *Verbascum* L. *In:* Heywood, VH, Tutin GT, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, Flora Europe **3**: 205-216 Cambridge.

Forey E, Barot S, Decaëns T, Langlois E, Laossi K R, Margerie P, Scheu S and Eisenhauer N 2011. Importance of earthworm-seed interactions for the composition and structure of plant communities: A review. Acta Oeco. **37**: 594-603.

Ghaderi FA, Soltani A and Sadeghipour HR 2008. Cardinal temperatures of germination in medical pumpkin (*Curcubita pepo* convar. *pepo* var. *styriaca*), borage (*Borago officinalis* L.) and black cumin (*Nigella sativa* L.). Asian J. Plant Sci. **7**(6): 574-578.

Heywood V H 1993. Flowering Plants of the World. Oxford University Press.

Huber-Morath A 1978. *Verbascum* L. *In:* Flora of Turkey and the East Aegean Islands. **6**: 461-603, Edinburg Univ. Press, Edinburgh.

Huber-Morath A 1981. *Verbascum* L. *In:* Rechinger KH., Flora Iranica **147**: 1-51, Akademische Druck-u Verlagsanstalt Graz-Austria.

Koger C H, Reddy K N, Poston D H 2004. Factors affecting seed germination, seedling emergence, and survival of texasweed (*Caperonia palustris*). Weed Science **52**: 989-995.

Kulkarni M G, Street R A and Van Staden J 2007. Germination and seedling growth requirements for propagation of *Dioscorea dregeana* (Kunth) Dur. and Schinz - A tuberous medicinal plant. S. Afr. Jour. of Bot. **73**: 131-137.

Martinez-Sanchez J J, Conesa E, Vicente M J, Jimenez A and Franco J A 2006. Germination responses of *Juncus acutus* (Juncaceae) and *Schoenus nigricans* (Cyperaceae) to light and temperature. Jour. of Arid Envir. **66**: 187-191.

Meikle R D 1985. *Verbascum* L. Flora of Cyprus, 2: 1196-1200, Published by The Bentham-Moxon Trust Royal Botanic Gardens, Kew.

Motsa M M, Slabbert M M, van Averbeke W and Morey L 2015. Effect of light and temperature on seed germination of selected African leafy vegetables. South Afr. Jour. of Bot. **99**: 29-35.

Sanchez-Romero C 2007. *In vitro* rescue of immature avocado (*Persea americana* Mill.) embryos. Sci. Horti. **111**: 365-370.

Tackholm V 1974. *Verbascum* L. Flora of Egypt p. 485-486, published by Cario University, printed by Cooperative Printing Company, Beirut.

Walter J, Jentsch A, Beierkuhnlein C and Kreyling J 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. Env. and Exp. Bot. **94**: 3- 8.

(Manuscript received on 21 June, 2016; revised on 18 October 2016)