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Effect of Temperature, Light, Seed Weight and GA_3 on the Germination of *Verbascum bithynicum*, *Verbascum wiedemannianum* and *Salvia dicroantha*

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Abstract: *S. dicroantha*, (Stapf in Denkseehr) *Verbascum bithynicum* and *V. wiedemannianum* are (Bioss.) endemic and medicinal potential plants disturbed in Turkey. *V. wiedemannianum* is different from the other *Verbascum* species in red-dark purple flowers and as a threated plant. Seed germination strategies of these species were studied with the aim of producing appropriate germination protocols for use ex situ conservation. The optimum temperature for seed germination of *S. dicroantha*, *V. bithynicum* and *V. wiedemannianum* was 20°C and darkness. The seeds of *V. bithynicum* and *V. wiedemannianum* incubated in darkness showed higher germination percentages than the seeds incubated with a 16:8 h photoperiod or continuous light, but the effect of application of darkness,photoperiod (16:8 h) or continuous light on the germination percentage of the seeds of *S. dicroantha* was not significant. In the case of *S. dicroantha*, seed weight significantly affected germination percentage, but not significant in the case of *V. bithynicum* and *V. wiedemannianum*. Exogenous GA_3 (20, 100, 200 mg L^{-1}) was comletely prevented germination the seeds of these species.

Key words: *Verbascum bithynicum*, *Verbascum wiedemannianum*, *Salvia dicroantha*, seed germination

INTRODUCTION

Salvia dicroantha, *Verbascum bithynicum* and *V. wiedemannianum* are endemic and medical plants disturbed in Turkey. *V. wiedemannianum* is different from the other *Verbascum* species in red-dark purple flowers and it has been listed in threatened species list (Ekim *et al*., 2000). It was known that the various parts of several *Verbascum* and *Salvia* species (leaves, stem, flowers etc.) had antioxidative and antimicrobial activity (Uçar *et al*., 2002; Tadeg *et al*., 2005; Tepe *et al*., 2006 a, b, c).

Detailed information on the different stages in the reproductive cycle of endemic, threatened and at the same time medicinal species may cotnribute to improved understanding of the phenomenon of endemism and at the same time assist conservation management decisions for the species under study (Navarro *et al*., 2003).

Each species has particular requirements for seed germination and germination requirements for native species are often unknown, particularly for rare or endemic species of which material is more difficult to obtain (Navarro *et al*., 2003; Cerabolini *et al*., 2004).

Temperature, light and gibberellic acid are the most important factors influencing the induction of seed germination (Baskin *et al*., 1998). In addition to these traits seed weight is an important factor for successful germination (Malcolm *et al*., 2003; Kambizi *et al*., 2006; Pérez-Garcia *et al*., 2006).

In this study, we report on the effects of temperature, light, GA_3 and seed weight on the germination of the seeds of these species.

MATERIALS AND METHODS

Seed collection: The capsules *V. bithynicum* and *V. wiedemannianum* and the nuts of *S. dicroantha* used for this study were harvested from their natural populations within Adana, Çorum and Samsun from Turkey, respectively in July-August of 2003. The seeds were removed from capsules and parts of perianths, air dried and stored in brown bottle at room temperature (18-25°C) until using time.

Germination tests: Seeds were soaked for 15 min in 1% NaOCl,washed with distilled water for three times and then placed in 9 cm petri dishes containing cotton and filter paper and moistured with 10 mL distilled water.

Temperature: The effect of temperatures on the germination were determined at 5, 10, 15, 20, 25 and 30°C.

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Light exposure: Seeds were incubated at 20°C in continuous dark (petri dishes were covered with aluminum foil) continuous light and 16:8 h (light:dark) photoperiod.

GA_3: Seeds were wetted with GA_3 in petri dishes (20, 100, 200 mg L^{-1}. lt concentrations) and then incubated at 20°C in continuous dark.

Seed weight: For each species 300 seeds were chosen at randomly. Each seed was weighed with a precision balance, and c assigned to two groups the seeds of *V. wiedemannianum* and *V. bithynicum* and three groups those of *S. dicroantha* (Table 1).

Data analysis:

Statistics: For evaluation of seed germination General Linear Model ANOVA procedure was used (SPSS 10.0 for Windows).

RESULTS

The results of this research showed that temperature and continuous dark affected the germination rate of *S. dicroantha*, *V. bithynicum* and *V. wiedemannianum* (Fig. 1).

The optimum temperatures for germination of the seeds of these species are 15-30°C.

The maximum germination percentage obtained at 20°C and continuous dark for *S. dicroantha*, *V. bithynicum* and *V. wiedemannianum* (49.44, 86.11, 86.66%, respectively).

For the seeds of *V. bithynicum*, the highest germination rate was obtained at 20-25°C and no significant difference ($p<0.01$) in germination was observed between seeds incubated at 20-25°C, but at 5°C the seeds was not germinated (Table 2).

The highest germination rate was observed at 20°C in *V. wiedemannianum* seeds at 5 and 10°C was not germination. There were statistically significant differences between studied temperatures ($p<0.01$). Also in the seeds of *S. dicroantha*, the highest germination rate was observed at 20°C, at 5°C no germination and the lest germination rate was observed at 10°C ($p<0.05$) (Table 2).

Continuous light and photoperiod (16:8 h) were decreased the germination rate of the seeds of *V. bithynicum* and *V. wiedemannianum*. The highest germination rate was occured in continuous dark (Fig. 2). There were statistically significant differences between studied temperatures ($p<0.05$). It was determined that the effects of continuous light, continuos dark and photoperiod were not significant on the germination of the seeds of *S. dicroantha* (Table 3).

Two different groups were formed according to seed weight from 300 random individuals of *V. bithynicum*, *V. wiedemannianum*. There were no significant differences between groups in terms of germination rate. Seeds of *S. dicroantha* were separated into

Table 1: The seeds groups according to seed weight. Seeds assigned to two groups the seeds of *V. wiedemannianum* and *V. bithynicum* and three groups those of *S. dicroantha*

	Groups		
Species	I (mg)	II (mg)	III (mg)
V. bithynicum	0.1-0.4	0.5-0.7	-
V. wiedemannianum	0.1-0.3	0.4-0.6	-
S. dicroantha	0.9-1.9	2.0-2.9	3-3.9

Table 2: The effect of temperature on the germination of *V. wiedemannianum*, *V. bithynicum* and *S. dicroantha*

Temp. (°C)	*V. wiedemannianum**	*V. bithynicum**	*S. dicroantha***
5	00.00±0.00d	00.00±0.00d	00.00±0.00e
10	00.00±0.00d	01.66±0.83c	01.11±0.0.73d
15	19.44±1.54c	01.66±1.17c	28.33±3.81bc
20	86.66±3.43a	86.11±3.20a	49.44±5.67a
25	57.77±4.25b	78.33±2.88a	32.77±2.51b
30	20.00±2.63c	46.11±1.61b	16.11±2.32c

*p<0.01, ** p<0.05, Each value represents germination percentage with standard error in parenthesis. Mean followed by the same letter are not significantly different at the 0.05 or 0.01 level

Table 3: The effect of continuous light, dark and photoperiod on germination

Parameters	*V. wiedemannianum***	*V. bithynicum**	*S. dicroantha**
Light	62.50± 3.59b	66.16±12.42a	43.33±3.57a
Photoperiod	64.16±8.30b	68.33±4.94a	45.00±3.65a
Dark	89.16±3.74a	90.00±2.58a	44.16±2.38a

*Not Significant, **p<0.05, Mean followed by the same letter are not significantly different at the 0.05 level

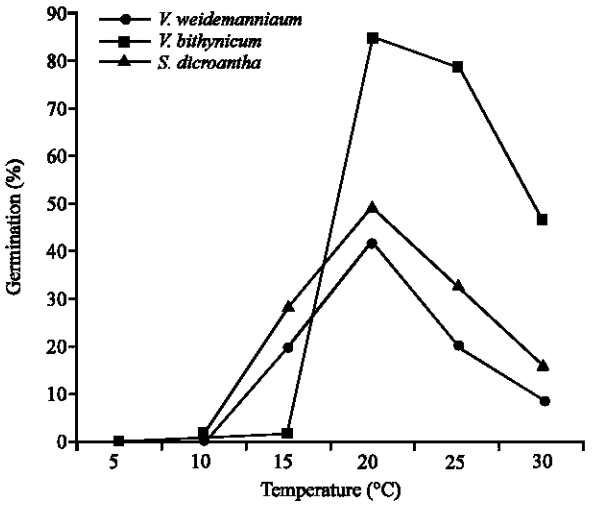

Fig. 1: The effect of temperature on the germination percentage

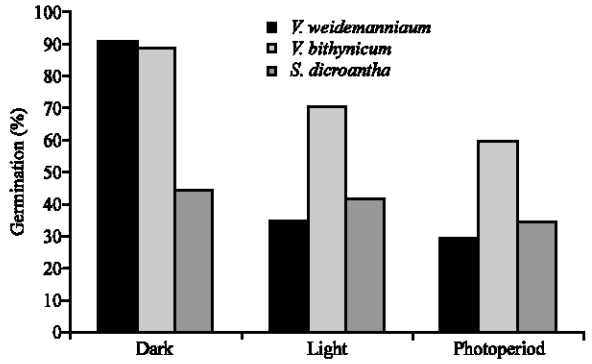

Fig. 2: The effect of continuous dark, light and photoperiod on germination of the seeds of *V. bithynicum, V. wiedemannianum and S. dicroantha*

three groups according to seed weight and significant differences were found between groups ($p<0.01$).

All applications of GA_3 were prevented germination of the seeds of three species (*V. bithynicum*, *V. wiedemannianum* and *S. dicroantha*).

DISCUSSION

The result of this research were indicated that conditions of temperature and light were affected germination of the seeds of three endemic species (*V. bithynicum*, *V. wiedemannianum* and *S. dicroantha*). At the lower of 20°C and the upper of 25°C, the germination percentage was reduced. The effect of temperature on the germination percentage was varied according to plant species (Leite *et al*., 2001).

Also in an other research the seeds of *V. olypicum*, *V. vbombyciferum* and *V. prusianum* were germinated at 4-7 °C (Saribayir, 2001).

The application of continuous light, dark and photoperiod (16:8 h light:dark) were affected germination of the seeds of three species in different level.

The germination percentage of the seeds of *V. bithynicum*, *V. wiedemannianum* was decreased in continuous light and photoperiod, however in the *S. dicroantha* was not observed significant difference between the light applications. It was determined that seeds of some species germinated to similar rates at light or dark, but those of other some species germinated to higher rates at dark than light. The seeds of *C. decidua*, *P. arabic* and *S. aegyptica* needed to light for germination (Huang *et al*., 2003), in *S. pomifera* white light increased to germination (Thanos *et al*., 1995), in *Cecropia hololeuca* increased to germination only at dark (Godoi *et al*., 2004), In *P. juliflora* germination in light was significantly greater than in the dark at high temperature (El-Keblawy *et al*., 2005). Also *P. grandiflora* and *P. viscosa* seeds germinated to higher rate at dark than 12:12 photoperiod (Navarro *et al*., 2003).

The results of this research and other researches were indicated that the effect of application light varied according to plant species.It is very important to determine appropriate temperature and light conditions for the best germination.

The application of GA_3 completely inhibited to germination in the research seeds. In the seeds of *E. hyemalis*, GA_3 not effected at 23°C, but at 4°C, GA_3 application stimulated germination (Tipirdamaz *et al*., 2000). The stimulating effect of GA_3 not universal and this effect varied according to germination conditions and plant species (Greipsson, 2001).

The seeds of *V. bithynicum* and *V. wiedemannianum* separated into two groups according to seed weight and no significant differences between groups in terms of germination rate. But *S. dicroantha* seeds separated into three groups and significant differences were found between groups ($p<0.01$).

It was determined that seed weight affected on the germination percentage and seedling growth (Arunachalam *et al*., 2003). Navarro *et al*. (2003) suggest that the poorer performance of lighter seeds is due to their lower endosperm content. Our results from *S. dicroantha* are in accordance with this hypothesis. The heavier seeds of *S. dicroantha* showed significantly greater germination rate than lighter seeds.

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