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Research Note

The Effects of Temperature and Gibberellic Acid on Germination of *Eranthis hyemalis* (L.) Salisb. seeds

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Abstract: In this study, the effects of different temperatures and exogenous gibberellic acid (GA_3) treatments on the germination of *Eranthis hyemalis* (L.) Salisb. seeds were studied. *E. hyemalis* seeds were exposed to the germination process at +4 °C and +23 °C in distilled water (control) and 0.10 , 5 and 10 mM concentrations of gibberellic acid (GA_3). While no germination was observed at +23 °C either in the control group or in the germination dishes where GA_3 was applied, at +4 °C, both in the control group (except for the 60th and 75th days) and with the all concentrations of GA_3, seed germination was observed. At +4 °C, all concentrations of GA_3 caused germination one month before germination occurred in to the control group.

Key Words: *Eranthis hyemalis*, germination, gibberellic acid (GA_3), temperature.

Eranthis hyemalis (L.) Salisb. Tohumlarının Çimlenmesi Üzerinde Sıcaklık ve Gibberellik Asit'in Etkisi

Özet: Bu çalışmada, *Eranthis hyemalis* (L.) Salisb. tohumlarının çimlenmesi üzerinde sıcaklık ve dışsal gibberellik asit (GA_3) uygulamasının etkisi çalışılmıştır. *E. hyemalis* tohumları +4 °C ve +23 °C'de distille su (kontrol) ve 0.10, 5 ve 10 mM konsantrasyonlarda giberellik asit (GA_3)'de çimlendirilme işlemine maruz bırakıldı. +23 °C'de kontrol grubunda ve GA_3 uygulaması yapılmış çimlendirme kaplarında çimlenme gözlenmezken, +4 °C'de hem kontrol grubunda (60. ve 75. günler hariç) ve hem de GA_3'ün bütün konsantrasyonlarında tohum çimlenmesi gözlenmiştir. +4 °C'de GA_3'ün tüm konsantrasyonları kontrol grubu ile karşılaştırıldığında bir ay öncesinden çimlenmeye neden olmuştur.

Anahtar Sözcükler: *Eranthis hyemalis*, çimlenme, gibberellik asit (GA_3), sıcaklık.

Introduction

Eranthis Salisb. *(Ranunculaceae)* is a perennial herb with tuberous rhizomes. This genus has 7 species native to Europe and Asia (1), but in Turkey (2) it is represented only by *Eranthis hyemalis* (L.) Salisb. (winter aconite). This plant is one of the most important commercial and export geophytes in Turkey (3). The collection of flower bulbs and rhizomes from wild populations is increasing rapidly (4).

Zimmer and Girmen stated that the export of *E. hyemalis* buds and tubers from Turkey is endangering the genetic resources of the region (5). *Eranthis stellata* Maxim. is also considered to be endangered in Tokuyosan National Park (6).

It has been shown that *E. hyemalis* seeds are immature when the dispersal unit is released (7), while some other studies point out that the seeds must be sown just after they have been collected (3), otherwise the seeds enter dormancy and can only be germinated the following year.

The aim of this study was to examine the effect of different temperatures and exogenous GA_3 treatments on the germination of *Eranthis hyemalis* seeds.

Material and Methods

E. hyemalis plants and seeds were collected by Prof. Dr. Ali Demirsoy (Hacettepe University, Faculty of Science, Biology Department, Beytepe, Ankara) from B3, Afyon, Dazkırı, from the bottom of the forest and open forest, Serpentine, 1500 m, 15, 05, 1995.

Seed germination procedure

The *E. hyemalis* seeds were surface sterilized by prewashing with distilled water and were then soaked in 96 % alcohol for three minutes, soaked in 30% of sodium hypochloride for 20 minutes, and washed with sterile distilled water 3 times for 5 minutes. The sterile seeds were soaked in different experimental solutions

(distilled water, 0.10, 5 and 10 mM GA_3) for 24 hours. The following day, 100 seeds were placed in germination dishes lined with a thin layer of cotton and two sheets of filter paper moistened with 20 ml of the different experimental solutions, as in the control group (distilled water, 0.10, 5 and 10 mM GA_3).

For germination, the dishes were left in a growth chamber at +23 °C and in a refrigerator at +4 °C in continuous darkness. After sowing, the germination percentages of the different treatments were determined after 60, 75, 90, 105 and 120 days. The experiment was performed with three replication.

Results and Discussion

The effects of 0.10, 5 and 10 mM GA_3 concentrations on the germination of *E. hyemalis* seeds were studied at +4 °C and +23 °C. No germination was observed at +23 °C either in the control group or in the germinating dishes where GA_3 was applied.

The differences in the germination percentages of *E. hyemalis* seeds in distilled water and three different concentrations of GA_3 at +4 °C are shown in Table 1.

While no germination was observed after 60 and 75 days in the control group, after 90, 105 and 120th days, the germination percentage was lower in the control group than with the GA_3 treatments at + 4 °C, i.e., all concentrations of GA_3 enhanced germination (Table 1). In all of the GA_3 treatments, germination had begun on the 60th day, and after 90, 105 and 120 days, germination amounted to 87% , 84% and 81% respectively. The effect of different concentrations of GA_3 on the germination percentage was nearly the same. In the control group, germination had begun by the 90 th day (2%) and was up to 76 % on the 120 th day. Germination began one month earlier in the GA_3 treatments than in the control group.

Table 1. The effect of three different GA_3 concentrations on the germination percentage of *E. hyemalis* seeds at +4 °C after 60, 75, 90, 105 and 120 day periods.

	Days				
Treatment	60	75	90	105	120
Distilled water (Control)	0	0	2	34	76
0.10 mM GA_3	12	56	87	87	87
5 mM GA_3	3	51	84	84	84
10 mM GA_3	7	48	81	81	81

It has been observed that if seeds are not sown just after they have been collected they become dormant (3). According to Bewley and Black (7), embryos of *E. hyemalis* are morphologically immature when released and require a period of further development before they are able to germinate. In some species which have morphologically immature embryos, the developmental period is followed by a period of embryo dormancy ended by chilling. In other species, to terminate dormancy a further 2-4 month chilling period (+5-10 °C) is required. The development of immature embryos in the seeds of *Heracleum spondylium* occurs best at low temperatures (+2-3 °C). The hydrated seeds of many woody and herbaceous species are released from dormancy when they experience relatively low temperatures from 1-10 °C, but species differ as to the most effective range. Recorded optima for most species are close to 5 °C which, for greatest effect, needs to occur over periods ranging from a few days to several months. The required chilling period is also related to the depth of dormancy.

The action of low temperatures in terminating dormancy may be: to promote a fall in the level of inhibitors, to increase the level of promotive hormones, or to increase their capacity for production. These events may occur in combination (7).

No germination was observed during the experimental period at +23 °C, either in the control group or in the germination dishes where GA_3 was applied. This was also the case at the low temperature (+4 °C) after 60 and 75 days in the control group. That there was no germination in the control group until the third month shows that there was probable dormancy in the seeds of *E.hyemalis.* On the other hand, the GA_3 treatments initiated germination a month earlier than in the control group. Our results clearly show that the dormancy was ended by GA_3 applications. It has been reported by other researchers that GA_3 are successful in ending dormancy in numerous species of seeds and also in accelerating the germination of non-dormant seeds. Effective concentrations generally lie within the range 0.10 - 10mM (7). In our results, 0.10 , 5 and 10 mM GA_3 concentrations affected germination but the variations in these effects were not found to be significant. As a result of hormonal changes, the seed becomes dormant. Essentially the events of dormancy and germination are controlled by a balance between hormones.

It has been reported that treatment with GA_3 can increse the formation of rough endoplasmic reticulum and polyribosomes (8). Moreover, it has been found that GA_3 stimulates the synthesis of mRNA which is specific for α-

amilase (9). As can be seen, GA_3 has the potential to regulate germination in numerous ways. The elaboration of an endomembrane system and the synthesis of new classes of mRNA can serve to regulate the synthesis of proteins required for germination.

In conclusion, in accordance with the literature the application of the low temperature (+4°C) ended the dormancy of *E.hyemalis* seeds in the third month, and GA_3 applications at +4°C caused germination one month before germination occurred in the control group.

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