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BREAKING OF SEED DORMANCY IN HALOPHYTIC ENDEMIC *SAPONARIA HALOPHILA* HEDGE & HUB.- MOR.

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Abstract

Saponaria halophila Hedge & Hub.-Mor. is an endemic plant species rarely spread throughout Turkey. This study was conducted to find out an appropriate method for breaking dormancy of seeds. Following sulphuric acid treatments, the seeds were exposed to six different doses of five plant growth regulators (PGR) BA, IAA, Kn, GA_3, NAA at various temperature regimes (10 - 15, 15 - 20, 20 - 25, 25 - 30 and 30 - 35°C) for 12 hrs light-dark photoperiods. The effects of various doses of different PGRs on germination percentages at certain temperatures were compared. The highest germination ratios for GA_3, NAA, IAA, Kn, BA were respectively observed as 83.3% at 50 ppm and 20 - 25°C, 75% at 400 ppm and 20 - 25°C, 65% at 50 ppm and 15 - 25°C, 55% at 25 ppm and 15 - 20°C, and 40% at 50 ppm and 20 - 25°C.

Introduction

Seed dormancy and germination are complicated adaptive traits of higher plants. These traits are affected by a large number of genes and environmental factors (Koornef *et al.* 2002). Although suitable environmental conditions required for germination are available, seeds of many plants species cannot germinate. Seed dormancy is caused by hard and impermeable seed coat and presence of immature or dormant embryo (Olmez *et al.* 2008). Seed dormancy is classified as physiologic, morphologic, morpho-physiologic, physical and combined dormancies. One of the most common types of dormancies is physical dormancy among the plant species. The hard and water-impermeable seed coats lead to physical dormancy which is observed in 16 families of angiosperms (Baskin *et al.* 2000, Baskin and Baskin 2004). The scarification techniques (treatment with acid, sand paper, removal of seed coat etc.) are commonly used in breaking physical dormancy of the seeds (Patane and Grestab 2006, Keshtkar *et al.* 2008, Tavili 2014). In breaking physiological dormancy, on the other hand, various PRGs (GA_3, IAA, Kn, NAA and BA etc.) are also used (Bakrim *et al.* 2007, Roychowdhury *et al.* 2012, Dhoran and Gudadhe 2012, Kaya *et al.* 2014).

The genus *Saponaria* L., belongs to *Caryophyllaceae*, commonly known as soapworts and is distributed throughout temperate Eurasia, generally in the Mediterranean and Irano-Turanian regions with approximately 40 species (Mabberley 2008). The genus *Saponaria* is represented by 27 taxa of which 12 are endemic in flora of Turkey (Güner *et al.* 2012). Most species of *Saponaria* have been growing in open areas and steppe in Turkey (Dönmez 2009). *S. halophila* is a halophytic endemic plant species that is found in saline and alkaline lands around the town of Karakol near Tuz Gölü (Lake Salt) in the province of Konya. This species spreads over a very small area and today it is an endangered species.

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The present study was conducted to determine the conditions required for the germination of the seeds of *S. halophila* since it is an endangered species due to climate change and overgrazing and has both seed coat and physiological dormancy. Identification of eco-physiological properties of this species with this study will probably provide significant outcomes for further taxonomic and auto-ecology studies.

Materials and Methods

The seeds of *Saponaria halophila* were collected from the salty steppes around the town of Karakol near Tuz Gölü (Konya) in July 2010 (38˚27'850" N - 33˚14'286" E, altitude 945m.) Before the experimentation, the type of dormancy of *S. halophila* seeds was determined. To this end, two pre-treatments were performed. In the first pre-treatment, 25 seeds whose surface sterilization was performed were transferred to Petri dishes containing 7 ml deionized water and were left to germinate in the incubator at 24°C for 10 days. At the end of the set period, it was determined that none of the seeds had germinated. Their first weights and their weights after 10 days were measured and it was observed that they had not absorbed any water. Such findings revealed that seeds had water-proof and hard coat structure. In order to thin the coats of the seeds and make them water-pervious, they were subjected to concentrated H_2SO_4 treatments for different durations (2, 4, 8 and 10 min). It was found that the seeds that were kept in concentrated sulfuric acid for 10 min germinated more than the ones in the control group.

In the second pre-treatment, whether or not the seeds had physiological dormancy were determined. To this end, first, the seeds were kept in concentrated sulfuric acid for 10 min and divided into 3 groups. The seeds of the control group were kept in deionized water whereas the seeds of the other two groups were respectively kept in 200 and 400 ppm GA for 24 hrs. At the end of the set period, the seeds were transferred to petri dishes and they were left to germinate for 10 days. In the meantime, the seeds that germinated were counted every other day. At the end of the set period, it was determined that the rate of germination was very low in the control group whereas it was very high in the seeds subjected to GA_3 treatments. Such results revealed that *S. halophila* seeds had both seed coat dormancy and physiological dormancy. Following the identification of the type of dormancy of *S. halophila* seeds, 6 different concentrations (25, 50, 100, 200, 400, 800 ppm) of 5 different plant growth regulators (BA, IAA, Kn, GA_3 and NAA) were tested in 5 different alternating temperature regimes (10 - 15, 15 - 20, 20 - 25, 25 - 30 and 30 - 35°C) in a 12 hrs light and 12 hrs dark photoperiod.

While the experiment was being set up, large and similar seeds were selected and kept in concentrated H_2SO_4 for 10 minutes and at the end of the period surface sterilization was performed using 1% sodium hypochlorite. Then, the seeds were transferred to plates that contained sterilized plant growth regulators of different concentrations while the seeds in the control group were put in dishes that had deionized water and were kept at +4°C for 24 hrs. At the end of the period, the seeds were placed in groups of 25 into Petri dishes that contained 7 ml of deionized water and were left to germinate at 5 different alternating temperature regimes in incubators providing 12 hrs light/12 hrs dark environments for 20 days. The germinated seeds were counted every other day. In order for a seed to be considered germinated, it was stipulated that the radicle should come out of the hilum section of the seed.

Germination percentages, germination rate indexes (GI), mean germination times (MGT) and germination rates (GR) of the seeds were calculated at the end of the experiment. MGT and GR were calculated using Ellis and Roberts's (1981) equations. The index of germination rate (GI) was estimated by using a modified Timson's (Khan and Ungar 1996) index of germination velocity. Experiments were conducted in 3 repetitions. The resultant data were analyzed by using

the one way analysis of variance technique (One-way ANOVA) and the differences among means were compared using the Tukey test. Significance level was taken as $p < 0.05$. Statistical analyses were performed with SPSS software (standard version 13.0).

Results and Discussion

10 - 15°C: Germination rates of the seeds subjected to plant growth regulator (BA, IAA, Kn, GA_3 and NAA) pre-treatments were generally higher than those of the seed of the control group. The highest germination rate (43.33%) was found in seeds treated with 200 ppm IAA (Fig. 1A). The lowest germination (3.33%) was observed in the seeds treated with 200, 400, 800 ppm Kn. The lowest MGT value was found to be 5.03% in the seeds treated with 800 pp GA_3 (Fig. 1B).

15 - 20°C: It can be stated that the most effective temperature in breaking seed dormancies of *S. halophila* was 15 - 20°C temperature regime. Germination percentage, GI and GR values at all plant growth regulator treatments were found to be quite high, while MGT value was quite low when compared to the control group. While the germination of the control group at 15 - 20°C temperature range was 3.3%, the highest germination (75%) was obtained from the seeds treated with 800 ppm GA_3 (Fig. 2A). The highest GI, GR values and the lowest MGT value at this temperature regime were respectively found to be 26.73, 0.24 and 4.17 in 800 ppm GA_3 treatments (Fig. 2B).

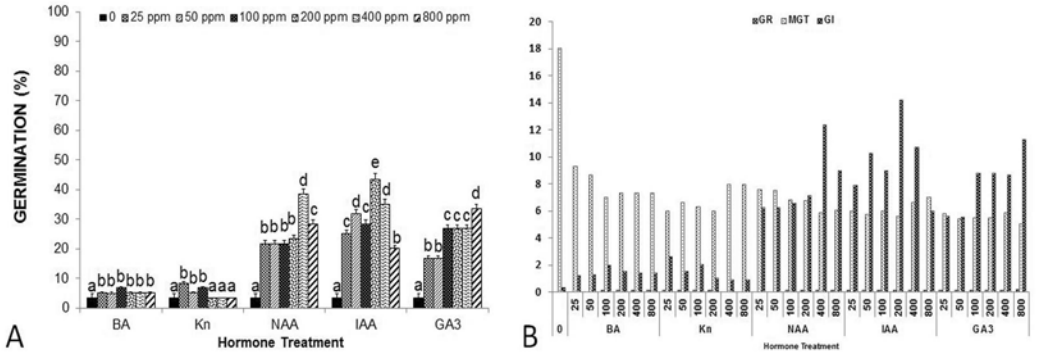

Fig. 1. The effects of different hormone treatments on *S. halophila* seeds at 10 - 15°C temperature regime A. The germination percentage B. GI, GR and MGT values. Vertical bars indicate ± standard error (SE). Different letters are statistically significant at the $p < 0.05\%$ level as analysed by Tukey test.

20 - 25°C: Whereas germination rate of the control group at 20 - 25°C temperature regime was 6.67 %, compared to control group, significant increases were observed in all the groups where plant growth regulator applications. The highest germination at this temperature range (83.33%) was determined in groups with 800 ppm GA_3 treatments (Fig. 3A). The highest GI, GR values and the lowest MGT value were also determined in the same group (Fig. 3B). The lowest germination among the plant growth regulators at this temperature was observed in groups with 800 ppm BA and Kn treatments (Fig. 3A).

25 - 30°C: Although germination rate was 6.67% at 25 - 30°C temperature regime in groups without hormone treatments, increases took place in germination rates of the treatment groups except for 800 ppm BA, 400 and 800 ppm Kn treatments (Fig. 4A). The highest germination rate (56.67%) at 25 - 30°C temperature regime was determined in groups with 200 ppm IAA treatment. The lowest MGT (4.10%) value was observed in groups with 400 ppm GA_3 treatment (Fig. 4B).

30 - 35°C: It was determined that germination of seeds subjected to hormone pre-treatments were generally higher than those of the seeds of control group. At 30 - 35°C temperature regime, the highest germination was determined to be 50% in groups with 200 ppm IAA treatments (Fig. 5A). On the other hand, the lowest MGT and the highest GR value was observed in the seeds treated with 800 ppm GA_3 (Fig. 5B).

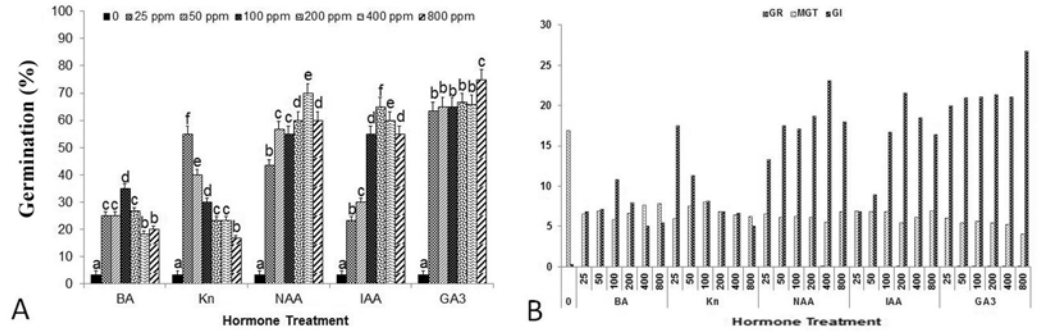

Fig. 2. The effects of different hormone treatments on *S. halophila* seeds at 15 - 20°C temperature regime A. The germination percentage B. GI, GR and MGT values.

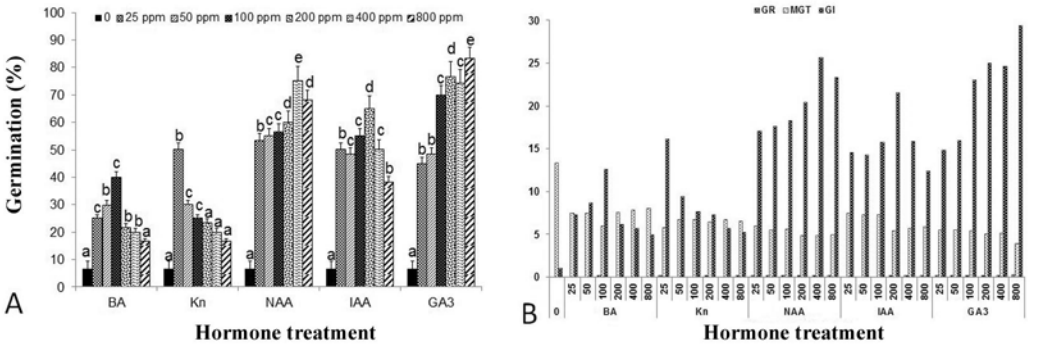

Fig. 3. The effects of different hormone treatments on *S. halophila* seeds at 20 - 25°C temperature regime A. The germination percentage B. GI, GR and MGT values.

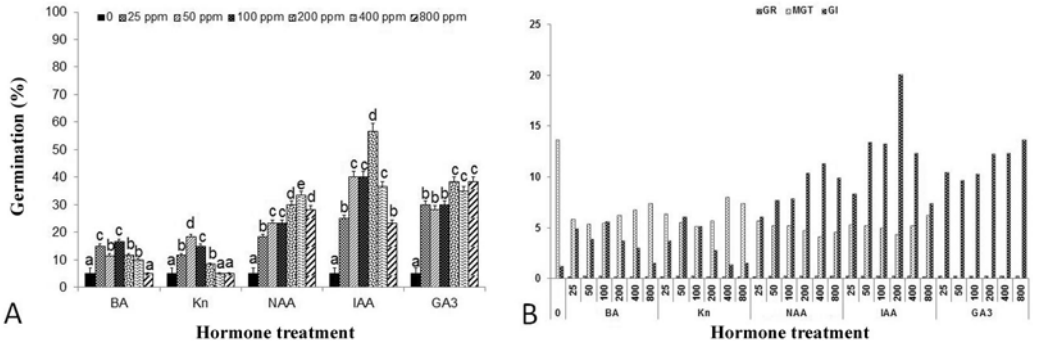

Fig. 4. The effects of different hormone treatments on *S. halophila* seeds at 25 - 30°C temperature regime A. The germination percentage B. GI, GR and MGT values.

The success of plant populations depend especially on the germination response of their seeds under various environmental conditions. Halophytes are salt-resistant or salt-tolerant plants that thrive and complete their life cycles in soils or waters containing high salt concentrations. The halophytes have several physiological adaptations that facilitate their survival in saline environments. The most common of them is ability of osmotic adjustment (Khan and Gul 2006). Several factors (salinity, light, temperature and water) interact in the soil surface, which regulate seed germination. Variation in temperature under saline conditions has different effects on germination of halophytes and this variation could be due to ecological regions of the world where they live (Ungar 1995, Khan 2003). Germination of halophytes in subtropical arid environments occurs when the soil salinity is substantially reduced by rains (Khan 1991).

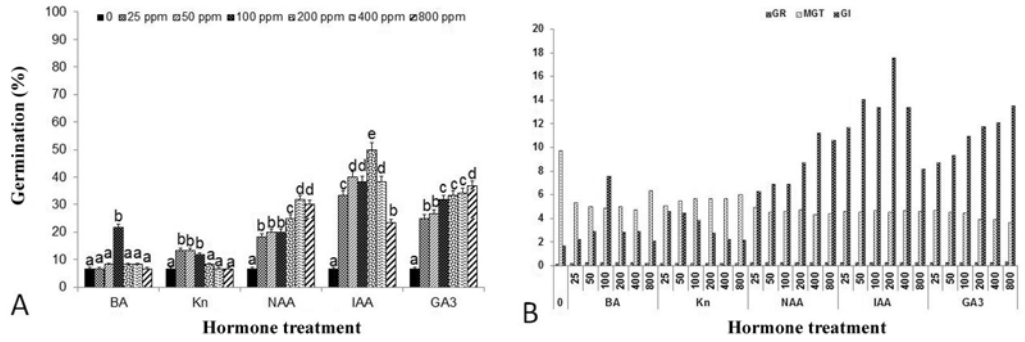

Fig. 5. The effects of different hormone treatments on *S. halophila* seeds at 30 - 35°C temperature regime A. The germination percentage B. GI, GR and MGT values.

Physical dormancy was observed in *S. halophila* seeds due to hard and impermeable seed coat. In order to physical dormancy, they were subjected to concentrated H_2SO_4 treatments for different durations. Similarly, it was reported that chemical scarification techniques were an effective method in breaking physical dormancy (Aliero 2004, Soyler and Khawar 2006, Suleiman *et al.* 2008, Yıldıztugay and Kucukoduk 2012). Germination rate of GA_3 treated seeds were significantly higher than the germination rate of control seeds without any hormone treatments. Such results revealed that *S. halophila* seeds had also physiological dormancy. The effects of different plant growth regulators on germination of the seeds at various temperatures were found to be effective in breaking physiological dormancy in this study. Similar findings were also reported by previous studies (Ghahfaroki and Afshari 2007, Han *et al.* 2010, Uğurlu and Dönmez 2013).

With regard to BA treatments, current findings revealed the highest germination rate (40%) in 100 ppm BA treatments at 20 - 25°C. The decrease observed in germination rate of 800 ppm BA treatment might have resulted from the toxic impacts of such high doses on germination of seeds. The present findings are supported by the results of Godo *et al.* (2010) on germination rates of *Calanthe tricarinata* Lindl. BA treatments of the present study yielded the highest germination rate at 20°C.

Considering the Kn treatments, the highest germination rate (55%) was observed in 25 ppm Kn treatments at 15 - 20°C. In a previous study investigating the effects of pre-chilling and kinetin treatments on germination of *Capparis spinosa* L. var. *spinosa* L. and *Capparis ovata* Desf. var. *canescens* (Coss.) Heywood seeds, the highest germination rate was observed in 200 ppm Kn treatments at 20 ± 1°C (Kaya *et al.* 2014). Current results indicated that the ideal germination temperature was 20°C for Kn. Kn was also proved to have stimulatory effects on germination of

dehusked seeds of indica and Japonica rice (*Oryza sativa* L.) under aerobic and anaerobic conditions (Miyoshi and Sato 1997). Roychowdhury *et al.* (2012) reported decreasing germination rates with increasing Kn doses. Current findings are consistent with such previous findings.

With regard to NAA treatments, the highest germination rate (75%) was observed in 400 ppm NAA treatments at 20 - 25°C. Godo *et al.* (2010) investigated the effects of plant growth regulators BA and NAA on germination of *C. tricarinata* seeds and reported the ideal germination temperature as 20°C. Researchers also indicated that both NAA and BA had stimulatory effects of germination, but such effects of BA were more distinctive than the effects of NAA. Current findings also revealed the ideal germination temperature of BA and NAA treatments as between 15 - 25°C. Contrary to findings of Godo *et al.* (2010), germination rate in 400 ppm NAA treatment (75%) was much higher than the germination rate in 50 ppm BA treatment (40%). Therefore, it was concluded that NAA stimulated germination more than BA did. Similar to findings of earlier studies, NAA had an effect on germination rate as compared to control during light and dark periods (Kanmegne and Omokolo 2007, Dhoran and Gudadhe 2012).

It was found that the germination rates of seeds with IAA treatments at different concentrations and temperature regimes were much higher than those of the control group. Among the IAA pre-treatment doses, 200 ppm was found to be the most effective hormone dose on germination. Banerji (1998) investigated the effects of BA, GA_3 and IAA, treatments on germination of *Melia azedarach* seeds and reported significant effects of growth regulators on germination rates.

Considering GA_3 treatments, the highest germination rates were observed in 800 ppm GA_3 treatments at 20 - 25°C temperature regime. The best result in the removal of dormancy in *Ferula gummosa* Boiss. seeds was obtained in GA_3 application of 1000 ppm (Rahmana-Ghahfarokhi and Tavakkol-Afshari 2007). Likewise, dormancy and germination requirements in *F. gummosa* and *Teucrium polium* L. seeds were examined and it was determined that germination rates increased in both species in high concentrations of GA_3 (Nadjafi *et al.* 2006). Generally GA_3 alone is the most successful growth regulator in all cases (Kabar 1997). The role of GA_3 and Kn in reducing the dormancy of the seeds of *Zygophyllum simplex* L. in salt solutions of various concentrations was investigated. GA_3 (0.3 and 3 mM) and Kn (0.05 and 0.5 mM) were quite effective in breaking dormancy in saline conditions. It was reported that especially GA_3 treatments increased germination rate in all salt concentrations (Khan and Ungar 1997). These findings were consistent with the findings of the present study. Ultimately, it was concluded herein that GA_3 was the most effective growth regulator on germination rates.

Breaking the dormancy state of the seeds by using *in vitro* techniques is extremely important in order to ensure survival of the species. The most useful methods in breaking dormancy of *S. halophila* seed were determined for the first time in the present study. Current finding may provide significant contributions to the *ex situ* preservation of the species.

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