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Full Length Research Paper

Breaking of seed dormancy in *Hypericum leptophyllum* Hochst., an endemic Turkish species

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Seed germination requirements of the endemic Turkish species of *Hypericum*, *Hypericum leptophyllum* Hochst. were studied by performing some pre-soaking treatments with the aim of describing suitable germination protocols for use in *ex situ* conservation. Before placing the seeds in Petri dishes, they were soaked in 50, 100 or 150 ppm GA; 0.5, 1 or 1.5% H_2SO_4; 150 ppm GA + 0.5% H_2SO_4 solutions, tap water and 40, 50 or 60°C hot water for 30 min. To evaluate the effect of light on germination rate, the study was performed under both continuous illumination and darkness in a growth chamber. Light was found to be the most important factor in seed germination. In the presence of light, gibberellic acid (GA) and sulphuric acid (H_2SO_4) treatments in different doses increased germination rate significantly. The germination response to the pre-soaking treatments was discussed as a possible result of double dormancy involving partially dormant embryo and hard seed coat. This is the first report on the endemic species.

Key words: *Hypericum leptophyllum*, seed dormancy, gibberellic acid (GA), sulphuric acid (H_2SO_4).

INTRODUCTION

Hypericum is a large genus of herbs or shrubs including approximately 400 species which grow wild in arid and semiarid regions of the world (Cirak et al., 2010a; Bertoli et al., 2011). Species of this genus have been reported to use as traditional medicinal plants due to their wound-healing, bactericide, anti-inflammatory, diuretic and sedative properties for the last two hundred years (Cirak, 2006; Odabas et al., 2009). Turkey is an important centre for *Hypericum* genus with the presence of 89 species of which 43 are endemic (Davis, 1988). *Hypericum leptophyllum* Hochst. which grows in some dry stony or rocky and calcareous zones of central Anatolia is one of the endemic species from Turkish flora. Its stem is 20 to 60 cm in length, erect or prostrate and branching from the base. Leaves are 5 to 35 mm, oblong or linear to elliptic. Yellow flowers are numerous and do not include black-dots, like the leaves. Capsules are 5 to 10 mm in diameter, with dorsal vittae and lateral vesicles (Davis, 1988). To author's knowledge, there is no report on the endemic species.

Today, some *Hypericum* species such as *Hypericum perforatum* (Cirak et al., 2007a), *Hypericum brasiliense* (Abreu et al., 2003), *Hypericum androsaemum* (Valentao et al., 2003), *Hypericum scabrum* (Ayan et al., 2008), *Hypericum perfoliatum* (Cirak et al., 2007b), *Hypericum montbretii* (Cirak and Radusiene, 2007) and *Hypericum triquetrifolium* (Ayan and Cirak, 2008) have become a valuable commodity to wild-crafters who supply these species for the herbal industry due to their phytomedicinal properties and the market for only *H. perforatum* has exceeded $570 million worldwide annually (Sirvent et al., 2002; Camas et al., 2008). The increased market demand for *Hypericum* derived products has led to uncontrolled harvesting of plant materials from wild populations (Cirak et al., 2010b). As a result, the availability of wild plants from different *Hypericum* species in Turkish, flora is strongly limited (Cirak et al., 2009). Thus, the present study focuses on the determination of *ex situ* requirements for seed germination in *H. leptophyllum* by performing some pre-soaking treatments to promote germination as an initial

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step in its conservation.

MATERIALS AND METHODS

Plant materials

Seeds were harvested from *H. leptophyllum* plants collected from Yozgat province of Turkey and stored at 4 ± 2°C in sealed plastic bags until used for germination tests.

Experimental procedures

In preliminary testing, seeds placed in Petri dishes did not germinate effectively under normal laboratory conditions. The pre-soaking treatments used in the study were different gibberellic acid (GA) and sulphuric acid (H_2SO_4) doses, hot water and tap water. Before placing the seeds in Petri dishes, they were soaked in 50, 100 or 150 ppm GA; 0.5, 1 or 1.5% H_2SO_4 solutions, tap water, 40, 50 or 60°C hot water for 30 min. Seeds also were soaked in 150 ppm GA and then 0.5% H_2SO_4 solutions for 30 min to evaluate double effect of these chemicals (Cirak et al., 2004a). The treated seeds were placed in individual, sterilised Petri dishes containing moisture-retaining paper liners. Paper liners in the Petri dishes were kept moist throughout the germination period. To evaluate the effect of light on germination, the study was performed in growth chambers under both continuous illumination (1200 lux white light) and darkness. Temperature was set at 20°C, recommended temperature for germination in *H. brasiliense* and *H. perforatum* seeds (Bertelle et al., 2004). Germination was measured as a percentage, 20 days after the experiment was initiated. The seeds showing radicle emergence were recorded as “germinated” (Come, 1970).

Data analysis

The experimental design was a factorial randomized block arrangement with three replications with 100 seeds in each. Germination percentages from the original data were transformed for statistical analysis (arcsine of square root of percent germination x 0.01). The transformed data were analyzed using ANOVA and differences among treatments were tested using the Duncan Multiple Range Test (level of significance $P < 0.01$).

RESULTS

Germination responses of seeds to the pre-soaking treatments are shown in Figure 1. According to the results of variance analysis, all treatments had a significant effect on the germination rate of *H. leptophyllum* (Figure 1).

In general, seed germination was low in all the treatments and light was found to be most important factor affecting germination. Under darkness, germination was blocked and no presoaking treatment was effective in enhancing germination. In the presence of light as continuous illumination by 1200 lux white light, the highest germination was induced by 150 ppm GA + 0.5% H_2SO_4 treatment (25.60%), followed by the treatments of 1.5% H_2SO_4 (20.22%) and hot water at 40°C (16.00%). Although, their positive effect, GA and hot water treatments deteriorated germination at increasing doses. Together with untreated control, tap water treatment resulted in the lowest germination rates (2.00%).

DISCUSSION

Light has been recognized since the mid-nineteenth century as a germination-controlling factor (Baskin, 2004) and it is frequently found to be a requirement in plant species native to arid lands (Puppala and Fowler, 2003). In general, absence of light has a negative effect on germination in several *Hypericum* species such as *H. perforatum* (Cirak et al., 2004b), *Hypericum gramineum* (Ash et al., 1998), *Hypericum avicullariifolium* (Cirak et al., 2007c) and *H. brasiliense* (Bertelle et al., 2004). In the present study, the germination rate of *H. leptophyllum* seeds was higher in the presence of light for all treatments and under dark conditions none of treatments were effective to induce germination. Similar results were reported by Macchia et al. (1983), Thompson and Whatley (1984), Campbell (1985) and Cirak et al. (2004b) for *H. perforatum* seeds.

Seeds of many wild plants have hard seed coats which restrict water absorption by the embryo. Permeability may be improved by scarifying the seed coat by mechanical means (for example, clipping, abrasion or immersion in hot water) or chemically with strong oxidative agents (for example, sulphuric acid or sodium hypochlorite) (Abdallah et al., 1989). In the present study, hot water and H_2SO_4 treatments were found to be effective to induce seed germination for *H. leptophyllum*. The results indicate the presence of physical dormancy, related to hard seed coat and overcame by hot water or acid scarifications. Similar results were also reported for *Hypericum lydum, Hypericum tetrapterum* seeds (Cirak et al., 2006), *Prosopis ferox* (Baes et al., 2002), *Hyoscyamus niger* (Cirak et al., 2004a) and some legumes (Grouzis and Danthu, 2001).

Studies of genetics and physiology have shown the important roles of the plant hormones like abscisic acid and gibberellin in the regulation of dormancy and germination (Koornneef et al., 2002). Gibberellins comprise the class of hormones most directly implicated in the control and promotion of seed germination. These compounds occur at relatively high concentrations in developing seeds but usually drop to a lower level in mature dormant seeds (Schwachtje and Baldwin, 2004). Endogenously applied gibberellins can relieve certain types of dormancy, including physiological dormancy, photodormancy and thermodormancy acting as a substitute for low temperatures, long days, or red light (Seiller, 1998). In this study, GA_3 increased germination significantly depending on doses indicating the presence of physiological dormancy related to partially dormant embryo.

The germination enhancing effect of GA_3 was reported from the studies carried on other species of *Hypericum*

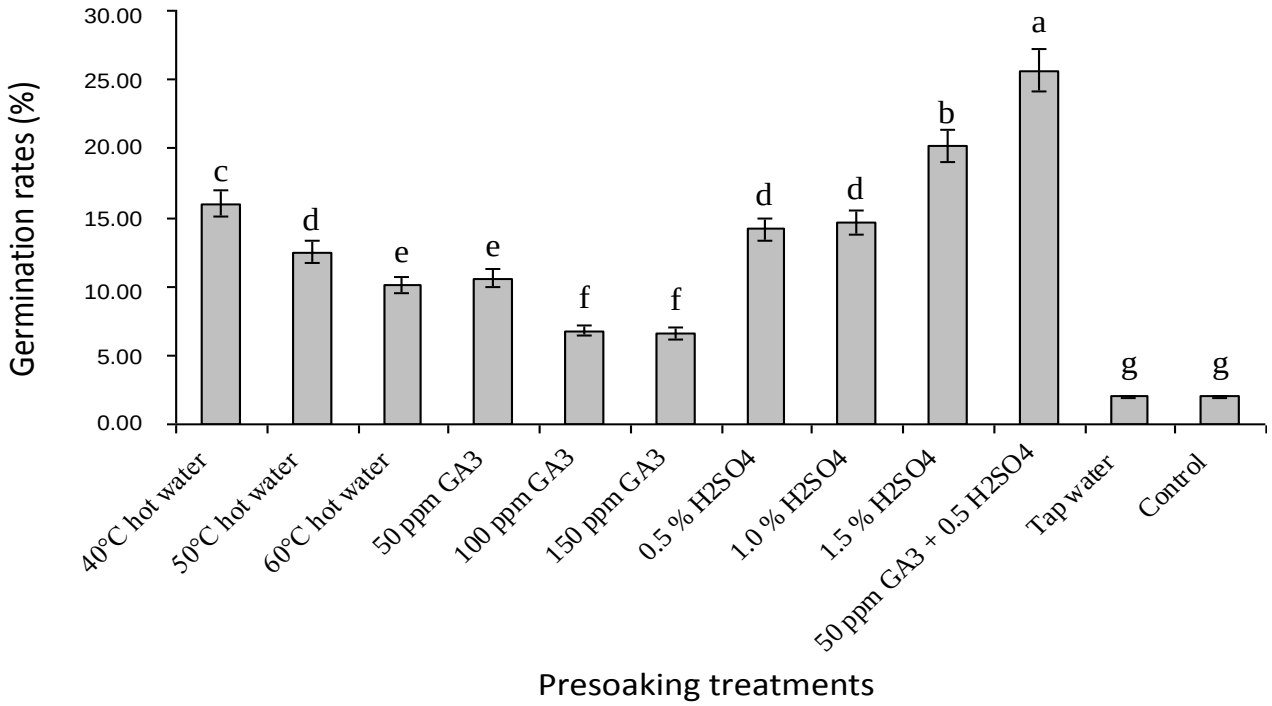

Figure 1. The germination rates of *H. leptophyllum* seeds exposed to different pre-soaking treatments under continuous illumination (Values with different letters within columns differ significantly at the level of $P < 0.01$; bars are ± S.E.).

such as *Hypericum androsaemum*, *H. scabrum* (Cirak et al., 2006) and *H. perfoliatum* (Cirak, 2007) as well as *Opuntia tomentosa* (Carrillo et al., 2003) and *Physoplexis comosa* (Cerabolini et al., 2004). It is interesting to note that 150 ppm GA + 0.5% H_2SO_4 treatment produced the highest germination rate in the present study leading us to believe the presence of double dormancy concerning hard seed coat and dormant embryo.

Chemicals that accumulate in the fruit and seed-coat during development and remain in the seed after harvest can act as germination inhibitors. Some of the substances associated with inhibition are various phenols, coumarin and abscisic acid which can be leached out by soaking in water (Booth and Sowa, 2001). In case of *H. perforatum* and *Hypericum avicullariifolium*, soaking the seeds in tap water resulted in a significant increase in germination (Cirak et al., 2004b and 2007c). Similarly, tap water treatment slightly increased germination rates in *Hypericum orientale* and *Hypericum pruinatum* seeds (Cirak, 2007). But in the present study, the same effect of water soaking was not observed and the treatment produced the lowest germination rate together with the untreated control.

Conclusions

Seed germination behavior is an integral part of *ex situ* conservation, especially for developing standard viability monitoring protocols and to ensure sufficient populations for germplasm regeneration. However, germination requirements for native species are often unknown. Here, it is the first time we have described the seed germination requirements of *H. leptophyllum*. Results from the present study indicate that the seeds exhibit double dormancy and have a district light requirement to germinate. Physical dormancy, which originated from hard seed coat could be eliminated effectively by a simple soaking in hot water and acid scarification. Physiological dormancy involving the presence of dormant embryo could be overcome by soaking in GA_3 solutions. Further studies based on field experiments to attain baseline data on *ex situ* plant development are currently underway.

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