Seed Germination of *Hypericum triquetrifolium* and *Hypericum heterophyllum*

Cüneyt Çirak • Ali Kemal Ayan • Mehmet Serhat Odabas*

University of Ondokuz Mayıs, Bafra Vocational School, 55400, Bafra, Samsun, Turkey

*Corresponding author* • mserhat@omu.edu.tr

**ABSTRACT**

In the present study, seed germination requirements of two Turkish *Hypericum* species, namely *H. triquetrifolium* and *H. heterophyllum* were studied by performing some pre-soaking treatments with the aim of describing suitable germination protocols. Before placing the seeds in Petri dishes, they were soaked in 50, 100 or 150 mg/L gibberellic acid (GA₃); 1, 2 or 3% H₂SO₄; tap water, 40, 50 or 60°C hot water for 30 min. The study was performed under a photoperiod of 18-h light/6-h darkness in growth chambers. In *H. triquetrifolium*, hot water 40°C (81%) and tap water (80%) treatments produced the highest germination rates followed by GA₃ 3100 mg/L (68%), GA₃ 50 mg/L (6%) and GA 150 mg/L (62%) treatments. Unlike the other applications, soaking the seeds in H₂SO₄ water 40°C (81%) and tap water (80%) treatments produced the highest germination rates followed by GA 3 100 mg/L (68%), GA 3 50 mg/L (6%) and GA 150 mg/L (62%) treatments. Unlike the other applications, soaking the seeds in H₂SO₄ solutions lowered seed germination when compared to the control. In *H. heterophyllum*, 2% H₂SO₄ was a unique treatment resulting in enhanced seed germination (20%). The variable germination responses are discussed as a possible result of dormancy involving the presence of a partially hard seed coat and chemical inhibitor(s) in *H. triquetrifolium* and a hard seed coat in *H. heterophyllum*.

**Keywords:** gibberellic acid, *Hypericum* L., seed dormancy, sulphuric acid, water soaking

**INTRODUCTION**

*Hypericum* is a genus of about 400 species of flowering plants in the family Guttiferae and the species of this genus have been used as traditional medicinal plants due to their wound-healing, bactericide, anti-inflammatory, diuretic and sedative properties for hundred of years (Demirci et al. 2005). In particular, extracts of *Hypericum perforatum* L. are now widely used in Europe as a drug for the treatment of depression (Patocka 2003). In Turkey, the genus is represented by 89 species of which 43 are endemic (Davis 1988).

*Hypericum triquetrifolium* Turra (Guttiferae) is an herbaceous perennial plant, which grows in open dry stony, sandy ground and cultivated fields in Turkey (Davis 1988). It has been used traditionally by Turkish folk in the treatment of bile and intestine ailments (Baytop 1999). The plant has great pharmaceutical potential with its well documented antinociceptive (Apaydin et al. 1999), anti-inflammatory (Ozturk et al. 2002), antioxidant (Conforti et al. 2002), antibacterial (Pistelli et al. 2005), antifungal (Fraternale et al. 2006) and cytotoxic (Conforti et al. 2007) activities. *Hypericum heterophyllum* Vent is an endemic species to Turkish flora. Although no traditional using in folk medicine was reported for *H. heterophyllum*, its extract was reported to exhibit antifungal activity (Cakir et al. 2004).

Germination is a critical stage in the life cycle of weeds and crop plants, and often controls population dynamics, with major practical implications (Keller and Kollmann 1999). Generally the germination capacity of *Hypericum* species is very low due to seed dormancy (Macchia et al. 1983) which in *H. perforatum* (Campbell 1985) and *H. aviculariifolium* (Cirak et al. 2007) is caused by a chemical inhibitor in the capsule. Plant growth regulators such as GA₃ (gibberellic acid) and IAA (indole-3-acetic acid) (Iglesias and Babiano 1997); chemicals such as H₂SO₄ (sulphuric acid) (Baes et al. 2002) and hot water treatments (Herman sen et al. 1999) have been recommended to break dormancy and enhance germination. A survey of the literature revealed that no study on seed germination of *H. triquetrifolium* and *H. heterophyllum* had been undertaken. Thus, the objective of this study was to determine the effect of exogenously applied GA₃, H₂SO₄ hot water and tap water on germination in finding effective methods for breaking the seed dormancy of aforesaid *Hypericum* species as an initial step in their domestication.

**MATERIALS AND METHODS**

**Plant materials**

A brief morphological description for the species examined was supplied in our previous studies (Ayan and Çirak 2006, 2008). The plant materials were identified by Dr. Hasan Korkmaz, Department of Biology, University of 19 Mayis, Samsun, Turkey. Voucher specimens were deposited in the herbarium of Ondokuz Mayis University Agricultural Faculty (OMUZF #127 for *H. heterophyllum* and OMUZF#134 for *H. triquetrifolium*).

**Experimental procedures**

The seeds were handpicked from at least 10 randomly selected *Hypericum* plants growing wild in the Çakalli district of Samsun province (41°04’N; 36°01’E); 470 m above sea level) and Erbaa district of Tokat province (40° 41’ N; 36° 34’ E); 230 m sea level), Turkey. The seeds were stored at 4 ± 2°C in sealed plastic bags until used for germination tests. 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 GA₃ and H₂SO₄ doses, hot water and tap water. Before placing the seeds in Petri dishes, they were soaked in 50, 100 or 150 mg/L GA₃; 1, 2 or 3% H₂SO₄ solutions, tap water, 40, 50 or 60°C hot water for 30 min. 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. The study was performed under a photoperiod of 18-h light/6-h darkness in growth chambers (Çirak et al. 2004). Temperature was set at 20°C, recom-
mended 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 Duncan’s multiple range test (*P* < 0.01).

**RESULTS AND DISCUSSION**

Seed responses to the pre-soaking treatments are shown in (Fig. 1, bottom). According to the results of variance analysis, the pre-soaking treatments tested had a significant effect, either positively or negatively, on germination rates depending on species. In *H. triquetrifolium*, hot water 40°C (81%) and tap water (80%) treatments produced the highest germination rates followed by GA3 100 mg/L (68%), GA3 50 mg/L (66%) and GA3 150 mg/L (62%) treatments. On the contrary, hot water treatments resulted in the highest germination rate significantly, depending on concentration in *H. triquetrifolium* when compared to control. Similar results were obtained in *H. perforatum* (Perez-Garcia et al. 2006) and *H. avicularrifolium* (Çirak et al. 2007). Hot water treatments have been reported to enhance germination of hard coated seeds by elevating water and O2 permeability of the testa (Aydın and Uzun 2001). In our case, hot water treatments resulted in the highest germination in *H. triquetrifolium* seeds. However, increasing degrees up to 40°C deteriorated germination greatly. The negative effect was probably due to the combination of high temperature and time, which may cause damage to the embryo tissue as observed in several species (Masamba 1994).

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 *avicularrifolium*, soaking the seeds in tap water resulted in a significant increase in germination (Çirak et al. 2004, 2007). In our case, tap water treatment significantly induced germination in *H. triquetrifolium* seeds.

Seed coat permeability may be improved by scarifying the seeds by chemically with strong oxidative agents (Abdallah et al. 1989). Likewise, high germination rates obtained with *H2SO4* solutions deteriorated seed germination when compared to control. As for *H. heterophyllum*, *H2SO4* 1.5% was the unique treatment resulting in enhanced seed germination (20%).

Light has been recognized since the mid-19th century as a germination-controlling factor and it is frequently found to be a requirement in plant species native to arid lands (Baskin 2004). In general, absence of light has a negative effect on germination in several *Hypericum* species such as *H. perforatum* (Campbell 1985), *H. gramineum* (Ash et al. 1998), *H. brasiliense* (Bertelle et al. 2004) and *H. avicularrifolium* (Çirak et al. 2007). In a previous study, we found that 18/6-h light/dark cycle was the most effective to meet light requirement for germination in *H. perforatum* seeds (Çirak et al. 2004). Thus, the present study was performed under this photoperiod to supply the probable light requirement.

Studies of genetics and physiology have shown the important roles of the plant hormones 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. 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, GA3 increased germination rate significantly, depending on concentration in *H. triquetrifolium* when compared to control. In conclusion, the present results indicated that seeds of both species of *Hypericum* exhibited physical dormancy. In *H. triquetrifolium* the dormancy was related to the presence of chemical inhibitor and partially hard seed coat and could easily be eliminated by soaking the seeds in either hot or tap water. On the contrary, germination was strictly restricted by hard seed coat and sulphuric acid scarification was found to be only effective treatment in eliminating the physical dormancy in *H. heterophyllum* seeds.

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