

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

Cüneyt Çırak • 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 100 mg/L (68%), GA₃ 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 (Fraternali *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* (Çırak *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 (Hermansen *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 Çırak 2006, 2008). The plant materials were identified by Dr. Hasan Korkmaz, Department of Biology, University of 19 Mayıs, Samsun, Turkey. Voucher specimens were deposited in the herbarium of Ondokuz Mayıs 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 Çakallı 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 (Çırak *et al.* 2004). Temperature was set at 20°C, recom-

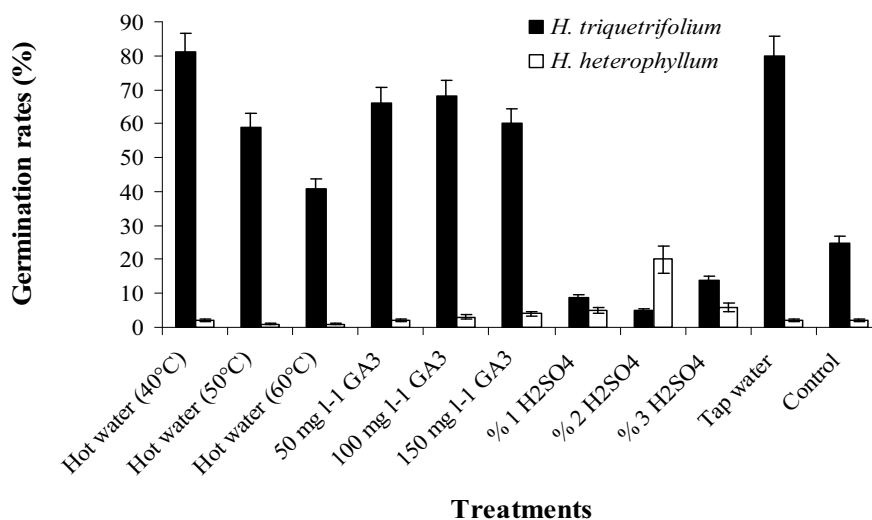


Fig. 1 The germination rates of *H. triquetrifolium* and *H. heterophyllum* seeds exposed to different pre-soaking treatments. (bars are \pm s.e.).

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 \times 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 of the other applications, soaking the seeds in 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. aviculariifolium* (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 thermodor-

mancy 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. Similar results were obtained in *H. perforatum* (Perez-Garcia *et al.* 2006) and *H. aviculariifolium* (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 *aviculariifolium*, 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 treatments were reported in some legumes (Grouzis and Danthu 2001), *Prosopis ferox* (Baes *et al.* 2002) and *Hyoscyamus niger* (Çirak *et al.* 2005) seeds. In the present study, H₂SO₄ scarification was found to be only treatment, effective in improving germination in *H. heterophyllum* seeds.

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.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. Dursun Ali Tokel, Faculty of Science and Arts, University of Ondokuz Mayıs, Turkey, for his technical assistance.

REFERENCES

- Abdallah MMF, Jones RA, El-Beltagy AS** (1989) An efficient method to overcome seed dormancy in Scotch broom (*Cytisus scoparius*). *Environmental Experimental Botany* **29**, 499-501
- Apaydin S, Zeybek U, Ince I, Elgin G, Karamenderes C, Ozturk B, Tuglular I** (1999) *Hypericum triquetrifolium* Turra. extract exhibits antinociceptive activity in the mouse. *Journal of Ethnopharmacology* **3**, 307-312
- Ash JE, Groves RH, Willis AJ** (1998) Seed ecology of *Hypericum gramineum*, an Australian forb. *Australian Journal of Botany* **45**, 1009-1022
- Ayan AK, Cırak C** (2008) Variation of hypericins in *Hypericum triquetrifolium* Turra growing in different locations of Turkey during plant growth. *Natural Product Research* **22**, 1597-1604
- Ayan AK, Cırak C** (2006) *In vitro* multiplication of *Hypericum heterophyllum*, an endemic Turkish species. *American Journal of Plant Physiology* **1**, 76-81
- Aydn I, Uzun F** (2001) The effects of some applications on germination rate of Gelemen Clover seeds gathered from natural vegetation in Samsun. *Pakistan Journal of Biological Sciences* **4**, 181-183
- Baes PO, Marta LV, Silvia S** (2002) Germination in *Prosopis ferox* seeds, effects of mechanical, chemical and biological scarifiers. *Journal of Arid Environments* **1**, 185-189
- Baskin CC** (2004) A classification system for seed dormancy. *Seed Sci. Res.* **14**, 1-16
- Baytop T** (1999) *Therapy with Medicinal Plants in Turkey*, Istanbul University Press, Istanbul, pp 66-167
- Bertelle FML, Beatriz PM, Augusto LA** (2004) Light, temperature and potassium nitrate in the germination of *Hypericum perforatum* L. and *H. brasiliense* Choisy seeds. *Bragantia* **63**, 193-199
- Booth DT, Sowa S** (2001) Respiration in dormant and non-dormant bitterbrush seeds. *Journal of Arid Environment* **48**, 35-39
- Cakir A, Kordali S, Zengin H, Izumi S, Hirata T** (2004) Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. *Flavour and Fragrance Journal* **19**, 62-68
- Campbell MH** (1985) Germination, emergence and seedling growth of *Hypericum perforatum*. *Weed Resources* **25**, 259-266
- Cırak C, Ayan A, Kevseroglu K, Caliskan O** (2004) Germination rate of St. John's wort (*Hypericum perforatum* L.) seeds exposed to different light intensities and illumination periods. *Journal of Biological Sciences* **4**, 279-282
- Cırak C, Kevseroglu K, Sağlam B** (2005) Physical and physiological dormancy in black henbane (*Hyoscyamus niger* L.) seeds. *Journal of Plant Biology* **47**, 391-395
- Cırak C, Kevseroglu K, Ayan AK** (2007) Breaking of seed dormancy in a Turkish endemic *Hypericum* species: *Hypericum aviculariifolium* subsp. *Depilatum* var. *depilatum* by light and some pre-soaking treatments. *Journal of Arid Environment* **68**, 159-164
- Come D** (1970) *Les Obstacles à la Germination*, Ed Masson, Paris, pp 10-11
- Conforti F, Loizzo MR, Statti AG, Menichini F** (2007) Cytotoxic activity of antioxidant constituents from *Hypericum triquetrifolium* Turra. *Natural Product Reports* **21**, 42-46
- Conforti F, Statti GA, Tundis R, Menichini F** (2002) Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia* **6**, 479-483
- Davis PH** (1988) *Flora of Turkey and the East Aegean Islands*, Edinburgh University Press, Edinburgh, pp 15-21
- Demirci B, Baser KHC, Crockett S, Khan IA** (2005) Analyses of the volatile constituents of asian *Hypericum* L. species. *Journal of Essential Oil Research* **17**, 659-663
- Fraternalia D, Bertoli A, Giamperi L, Bucchini A, Ricci D, Menichini F, Trinciarelli E, Pistelli L** (2006) Antifungal evaluation of *Hypericum triquetrifolium* polar extracts against *Fusarium* spp. *Natural Product Communications* **1**, 1117-1122
- Grouzis ASM, Danthu P** (2001) Seed germination of seven Sahelian legume species. *Journal of Arid Environment* **49**, 875-882
- Hermansen A, Brodal G, Balvoll G** (1999) Hot water treatments of carrot seeds: Effects on seed-borne fungi, germination, emergence and yield. *Seed Science and Technology* **27**, 599-613
- Iglesias RG, Babiano MJ** (1997) Endogenous abscisic acid during the germination of chickpea seed. *Physiologia Plantarum* **100**, 500-504
- Keller M, Kollmann J** (1999) Effects of seed provenance on germination of herbs for agricultural compensation sites. *Agricultural Ecosystems and Environment* **72**, 87-99
- Koornneef M, Bentsink L, Hilhorst H** (2002) Seed dormancy and germination. *Current Opinion in Plant Biology* **5**, 33-36
- Macchia N, Benvenuti A, Angelini L** (1983) Germination characteristics of some seeds of medicinal plants. *Rastitel'nye Resursy* **21**, 461-463
- Masamba C** (1994) Presowing seed treatments on four African *Acacia* species, appropriate technology for use in forestry for rural development. *Forest Ecology and Management* **64**, 105-109
- Ozturk B, Apaydin S, Goldeli E, Ince I, Zeybek U** (2002) *Hypericum triquetrifolium* Turra. extract exhibits antiinflammatory activity in the rat. *Journal of Ethnopharmacol* **80**, 207-209
- Patocka J** (2003) The chemistry, pharmacology, and toxicology of the biologically active constituents of the herb *Hypericum perforatum* L. *Journal of Applied Biomedicine* **1**, 61-73
- Perez-Garcia F, Huertas M, Mora E, Pena B, Varela F, Gonzalez-Benito ME** (2006) *Hypericum perforatum* L. seed germination: interpopulation variation and effect of light, temperature, presowing treatments and seed desiccation. *Genetic Resources and Crop Evolution* **9**, 1-12
- Pistelli L, Bertoli A, Morelli I, Menichini F, Musmanno RA, Di Maggio T, Coratza G** (2005) Chemical and antibacterial evaluation of *Hypericum triquetrifolium* Turra. *Phytotherapy Research* **19**, 787-791
- Seiller GJ** (1998) Seed maturity, storage time and temperature, and treatment effects on germination of two wild sunflowers. *Agronomy Journal* **90**, 221-226