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PHYSIOLOGICAL AND PHYSICAL SEED DORMANCY OF SOME *HYPERICUM* SPECIES GROWING IN TURKEY

ABSTRACT

To investigate the nature of dormancy in *Hypericum androsaemum, H. scabrum, H. lydum* and *H. tetrapterum* seeds which did not show germination under normal laboratory conditions. The 15 day long germination experiment under the constant light preceded by 30 min soaking in water (tap water, 40, 50 and 60°C hot water), GA_3 (50, 100 and 150 ppm) and H_2SO_4 (1, 2 and 3%) was performed. The germination response to the pre-soaking treatments was variable and discussed as a possible result of double dormancy involving partially dormant embryo and a chemical inhibitor for *H. androsaemum* and *H. scabrum*. Seed germination was restricted only by hard seed coat in *H. lydum,* and *H. tetrapterum* indicating the presence of physical dormancy.

Key words: double dormancy, germination, *Hypericum androsaemum, H. lydum, H. scabrum, H. tetrapterum,,* pre-soaking treatments

INTRODUCTION

Hypericum species are of great interest for mankind throughout many centuries and have been used as traditional medicinal plants due to their wound-healing (Yazaki and Okuda, 1990), bactericide (Ishiguro *et al.*, 1998), anti-inflammatory (Dias *et al.*, 1998), diuretic and sedative properties (Holz and Ostrowiski, 1987) for last two hundred years. The *Hypericum* genus of *Guttiferae* is represented in Turkey by 89 species of which 43 are endemic; the most abundant and well known is *H. perforatum* (Davis, 1988).

Hypericum androsaemum L. grows in West Europe (Flora Europaea, 1968) and in damp or shady places of Turkey (Davis, 1988). Infusion of *H. androsaemum* leaves has been used in traditional medicine for its diuretic and anti-hepatotoxic activities (Costa, 1994). Phenolic compounds, such as flavonoids, phenolic acids and xanthones, are known to be present in this species (Schmidt *et al.*, 2000) and have

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been reported to be responsible for its pharmacological properties (Valentao *et al.*, 2002). *Hypericum scabrum* L. is an herbaceous perennial plant which is distributed in dry rocky slopes and open woodland of Turkey (Davis, 1988) and has been used by Turkish folk for its antispasmodic, sedative and anti-inflammatory properties (Tanker, 1971). In Turkey, a skin remedy is prepared from its aerial parts and it is used as an ointment against psoriasis in folk medicine. Antibacterial activities of *Hypericum scabrum*have been reported by several *in vitro* studies (Azýrak and Erdođrul, 2003). Although there are several investigations on the phytochemistry and biological activity of *H. androsaemum* and *H. scabrum*, little effort has been dedicated to the study of other aspects of these species and there is, so far, no report related to *H. lydum* Boiss and *H. tetrapterum* Fries growing wild in rocky slopes and stream sides in Turkey. These species of *Hypericum* have not been domesticated yet in Turkey and due to uncontrolled harvesting as well as seasonal, climatic, and geographic restrictions, the availability of wild plants from their native populations is strongly limited.

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 germination capacity of *Hypericum* species is very low due to seed dormancy (Macchia *et al.*, 1983) which is caused by a chemical inhibitor exudate from the capsule in *H. perforatum* (Campbell, 1985) and absence of light has a negative effect on germination in *Hypericum* species (Çýrak *et al.*, 2004a). Plant growth regulators such as GA_3 (gibberellic acid) and IAA (indoleacetic acid) (Iglesias and Babiano, 1997); chemical substances such as H_2SO_4 (sulphuric acid) (Baes *et al.*, 2002) and hot water treatments (Hermansen *et al.*, 1999) have been recommended in breaking dormancy and to enhance germination. The objectives of this study were to determine the effect of exogenously applied GA_3, H_2SO_4, hot and tap water on germination and to find an effective method for breaking seed dormancy of *H. androsaemum, H.scabrum, H. lydum and H. tetrapterum*.

MATERIALS AND METHODS

The 10 month-old seeds of *H. androsaemum, H.scabrum, H. lydum and H. tetrapterum* wild plants growing in different places of Black Sea geographical region of Turkey were used as a material. In preliminary test, the seeds did not show germination under normal laboratory conditions (at 20 °C during 21 days (Anonymous, 2005)), so they were soaked in 50, 100 and 150 ppm GA_3; 1, 2 and 3% H_2SO_4 solutions, tap water, 40, 50 and 60°C hot water for 30 minutes and germinated on Petri dishes under continuous illumination (1200 lux white light) in growth chambers at 20°C. Tap water application was treated as control and the germination capacities were calculated as "percentage". Germination of the seeds exposed to different pre-treatments was evaluated 15 days later. Three replications (100 seeds each) of each pre-treatment were objected to ANOVA and differences among treatments were tested Duncan Multiple Range Test (Level of significance $P<0.01$).

RESULTS

The pre-soaking treatments tested in the present study had a significant ($P<0.01$) effect on germination depending on species (Figure 1).

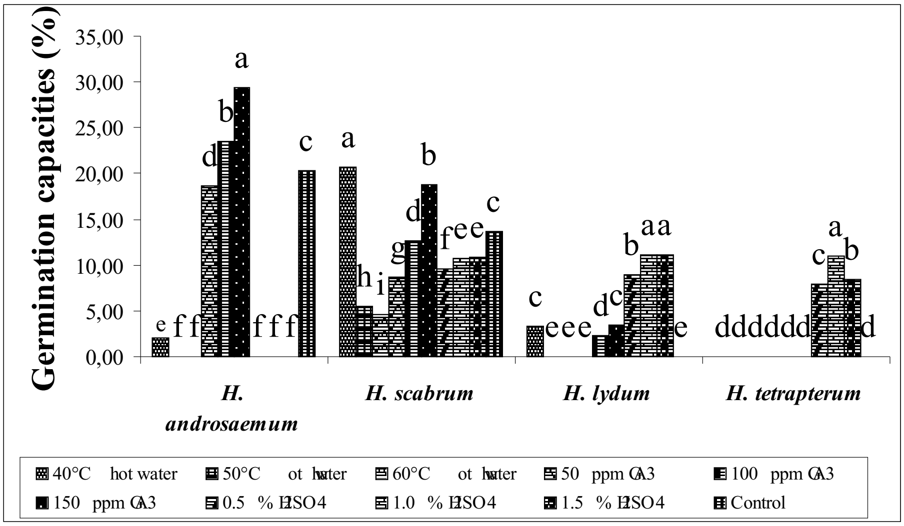

Fig. 1. The germination capacities of *H. androsaemum, H. scabrum, H. lydum* and *H. tetrapterum* seeds exposed to different pre-soaking treatments. (Values with different letters within columns for each species differ significantly at the level of $P<0.01$).

In *H. androsaemum,* GA_3 treatments were found to be most effective to improve seed germination while germination was deteriorated by hot water and H_2SO_4 when compared to control. 150 and 100 ppm GA_3 treatments gave the highest germination for this species with 29 and 24% respectively. These numbers were followed by control with 20%.

In contrast to the other *Hypericum* species evaluated in this study, germination response of *H. scabrum* seeds to hot water treatments was positive and significant. The highest germination for *H. scabrum* was obtained from 40 °C hot water treatment (21%) and this was followed by 150 ppm GA_3 and water-soaked control with 19 and 14% respectively. Also, when compared to control, H_2SO_4 treatments were ineffective to enhance germination.

H_2SO_4 treatments were the only effective ones in promoting germination for *H. lydum* and *H. tetrapterum* seeds. 2 and 3% H_2SO_4 treatments resulted in 11% germination in *H. lydum* and 11 and 8.37% germination in *H. tetrapterum* respectively. The other treatments were all generally ineffective in improving germination for both species.

DISCUSSION

Light has been recognized since the mid-nine-tenth century as a germination-controlling factor. In general, *Hypericum* species need light to germinate at the highest level (Ash *et al.*, 1998; Çýrak *et al.*, 2004a). Therefore, this study was conducted out under continuous illumination to supply this light requirement.

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 (e.g. clipping, abrasion or immersion in hot water) or chemically with strong oxidative agents (e.g. sulphuric acid or sodium hypochlorite) (Abdallah *et al.*, 1989). In the present study, H_2SO_4 treatments were found to be the most effective to induce seed germination for *H. lydum* and *H. tetrapterum*. The results indicate the presence of physical dormancy in both species related to hard seed coat and overcame by only acid scarification. However, it should be noted that the acid pre-treatment had a strong damage on seed coat in *H. androsaemum.* The high germination obtained with H_2SO_4 treatments agree with those obtained for other species like: *Prosopis ferox* (Baes *et al.*, 2002), *Acacia origena*, *Acacia pilispina* and *Pterolobium stellatum* (Teketay, 1998).

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 in *H. androsaemum* and *H. scabrum* indicating the presence of physiological dormancy related to partially dormant embryo for both species. The germination enhancing effect of GA_3 was reported from the studies carried on other species such as *Sesamum indicum* (Kyauk *et al.*, 1995), *Zea mays* and *Glycine max* (Wang *et al.*, 1996).

Previous researches with hot water treatments to induce germination were inconsistent and variable. Some of them have reported hot water treatments to enhance germination of hard coated seeds by elevating water and O_2 permeability of testa. Hermansen *et al.* (1999) reported that germination was enhanced by presoking in 44, 49 and 54°C hot water for 5-40 minutes in carrot seeds and dipping seeds in boiling water for 1–60 s. was very effective in promoting germination for *Dodonea viscosa* (Davis *et al.*, 2004). On the contrary, no significant seed germination response to hot water treatments has been reported for some *Acacia* species (Masamba, 1994) and *Myrtus communis* (Khosh-Khui and Bassiri 1976). Similarly, in the present study the effects of hot water treatments on seed germination of the *Hypericum* species tested were changeable and *H. scabrum* was the only species showing positive germination response to hot water treatments indicating the presence of physical dormancy.

Chemicals that accumulate in fruit and seed covering tissues during development and remain with the seed after harvest can be shown to act germination inhibitors. Some of the substances associated with inhibition are various phenols, coumarin and abscisic acid and can be leached out of the seeds by soaking in water. For example, in a previous study on *Hypericum perforatum* we observed that the chemical inhibitor in exudate from capsule could be eliminated effectively by a simple soaking in tap water (Çýrak *et al.*, 2004b). In this study, similarly, the colour of the water in which both seeds were soaked turned yellowish and water-soaked control resulted in higher germination in *H. androsaemum* and *H. scabrum*. The results suggested that seeds of both species include some chemical inhibitors can be leached by soaking in tap water.

CONCLUSION

Consequently, the results from the present study showed that the effects of different pre-soaking treatments on seed germination of tested *Hypericum* species were significant depending on species. GA_3 and water-soaked control were the most effective treatments to improve germination of *H. androsaemum,* suggesting that the seeds exhibit both physiological dormancy involving a partially dormant embryo and chemical dormancy associated with a chemical inhibitor. For *H. scabrum,* all treatments were generally effective in promoting germination. But hot water and GA_3 treatments increased germination mainly. It can be concluded that *H. scabrum* seeds, like those of *H. androsaemum*, exhibit double dormancy: the physical dormancy involving a hard seed coat and the physiological dormancy related to a partially dormant embryo. H_2SO_4 treatments were the only effective ones in promoting germination for *H. lydum* and *H. tetrapterum* seeds. In contrast to *H. androsaemum* and *H. scabrum*, seed germination was restricted only by hard seed coat in *H. lydum* and *H. tetrapterum* indicating the presence of physical dormancy.

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