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Nature of Seed Dormancy in Stinging Nettle (*Urtica dioica* L.)

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ABSTRACT

The present study was conducted to determine the germination requirements of stinging nettle seeds. For this purpose, before placing the seeds in Petri dishes, they were soaked for 30 min in 50, 100 and 150 mg/L GA solution, 40, 50 and 60°C hot water, or 0.5, 1 and 1.5% H_2SO_4 solutions. To evaluate the effect of light on germination, the study was performed in growth chambers under both continuous illumination [1200 lux (14.4 µmol/m^2/s) white light] and darkness. Light was found to be the most effective factor in promoting germination. Untreated control seeds under light produced the highest germination rate observed in the present study (83.33%) followed by light-mediated 30 and 40°C hot water treatments (44 and 38%, respectively). In contrast, all other treatments significantly deteriorated germination and this negative effect was more pronounced in H_2SO_4 treatments. The results indicated that European nettle seeds may have endogenous dormancy concerning their light requirement for germination.

Keywords: GA_3, H_2SO_4, hot water, light requirement, seed germination

INTRODUCTION

Stinging nettle (*Urtica dioica* L.) is a herbaceous flowering plant native to Europe, Asia, northern Africa, and North America, and is the best known member of the genus *Urtica* (Mabberley 1997). A total of 48 genera and 1050 species within the family Urticaceae were listed by Mabberley (1997). Those species consist of perennials and annuals with different biological properties. Nettle is a perennial species which flowers from June to August and spreads by seeds and by vegetative reproduction via creeping underground rhizomes. This plant produces copious seeds, 10,000 to 20,000 seeds per shoot. Seeds are elliptic in appearance and the 1000 seeds weight is 0.14-0.17 g (Cronquist 1981). Nettle has traditionally been used as a medicine against several diseases such as diabetes, eczema, hemorrhoid, anemia, rheumatism and prostate problems by many of different cultures in herbal medicine for over 2000 years (Tahri *et al*. 2000). This plant has a great pharmaceutical potential with its well documented analgesic (Yongna *et al*. 2005), antimicrobial (Uzun *et al*. 2004), antibacterial (Aksu and Kaya 2004), antidiabetic (Farzami *et al*. 2003), cardiovascular (Testai *et al*. 2002), diuretic (Tahri *et al*. 2000) and anti-inflammatory (Randall *et al*. 1999) effects. Stinging nettle has been receiving worldwide interest recently, not only for its pharmaceutical merit but because it is also hailed as one of the most important plants in high quality fiber production (Vogl and Hart 2002). Especially in Turkey, studies on nettle cultivation for the purpose of fiber production are on-going (Ayan *et al*. 2006) in which propagation via either *in vitro* culture or rooting of stem cuttings was inadequate in producing sufficient seedlings for establishing economical plantations.

Germination is the first and most critical stage in the life cycle of both wild and domesticated plants, and has major practical implications regarding the dynamics of plant communities (Keller and Kollmann 1999). Generally seeds of wild plant species exhibit different kinds of dormancy and germination are restricted by several external or internal factors such as a hard seed coat, chemical inhibitors, a partially dormant embryo or the absence of light (Çırak *et al*. 2004). Although dormancy has been studied exhaustively for the last three decades, the main principles for several types of dormancy remain unclear (Rehman and Park 2000). Nevertheless, several pre-treatments namely, plant growth regulators such as GA (gibberellic acid) and IAA (indoleacetic acid) (Iglesias and Babiano 1997); chemicals such as sulphuric acid (H_2SO_4) and hot water treatments (Tomer and Maguire 1989; Baes *et al*. 2002) have been recommended to break dormancy and enhance germination. To the authors' knowledge, there is no published report on the nature of seed dormancy in stinging nettle. Thus, the present study focuses on the determination of seed germination requirements of stinging nettle by performing some pre-soaking treatments to promote germination as an initial step in its large scale-up production.

Plant material

Stinging nettle seeds were collected from wild growing plants in the Samsun province of Turkey (41° 35′ N Lat., 35° 56′′ E Long., and 50 m elevation) in August 14-17, 2007 and stored at 4 ± 1°C in sealed plastic bags until used for germination tests.

Experimental procedures

In preliminary testing, the three-weeks old seeds placed in Petri dishes did not germinate effectively (lower than 30%) under normal laboratory conditions [20°C, under darkness, during 21 days (Anonymous 2005)]. The pre-soaking treatments used in the study were different doses of GA, hot water and H_2SO_4. Before placing the seeds in Petri dishes, they were soaked for 30 min in 50, 100 or 150 mg/L GA_3 solution, 40, 50 and 60°C hot water, or 0.5, 1 and 1.5 % H_2SO_4 solutions. The seeds left to germinate under both light and darkness without soaking in chemical and hot water solutions were treated as controls. All chemicals were purchased from Sigma Chemical Company (Germany). To evaluate the effect of light on germination, the study was performed in growth chambers under both continuous illumination [1200 lux (14.4 µmol/m^2/s) white light] and darkness. Temperature was set at 24°C (Çırak *et al*. 2004, 2006, 2007). The experimental design was a factorial randomized block arrangement with three replications with 100

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Short Communication

seeds in each. Germination was measured as a percentage, 20 days after the experiment was initiated. Germination percentages from the original data were transformed for statistical analysis (arcsine of square root of percent germination × 0.01). The transformed data were analyzed using ANOVA and differences among treatments were tested using Duncan's Multiple Range Test (level of significance: $P<0.01$).

RESULTS AND DISCUSSION

Seed responses to the pre-soaking treatments are shown in **Fig. 1**. According to the results of variance analysis, the pre-soaking treatments tested had a significant ($P<0.01$) effect on germination rates. Untreated control seeds under light produced the highest germination rate observed in the present study (83.33%) followed by light-mediated 30 and 40°C hot water treatments (44 and 38%, respectively). Light was found to be the most effective factor in promoting germination. In contrast, all other treatments significantly deteriorated germination when compared to control under both light and darkness. This negative effect was more pronounced in H_2SO_4 treatments.

Light has been recognized since the mid-nineteenth century as a germination-controlling factor and it is frequently found to be a requirement for wild plant species rather than domesticated ones (Baskin 2004). Light has a regulatory effect on germination and endogenous seed dormancy is usually related to the absence of light (Arechiga *et al*. 1997). For example, *Paulownia tomentosa* and *P. fortenii* seeds are light sensitive to a large extent. Especially the latter had an obligatory light requirement for germination and its seeds induced dormancy in darkness, which could be broken by exposing to red light (Dragoljub *et al*. 1985). In the case of *Halophila engelmannii*, seeds showed an increase in the rate of germination under increased light intensity and germination stopped after transfer to darkness, indicating a light requirement to break endogenous seed dormancy (McMillan 1987). Similarly, light requirement to germinate is very common among *Hypericum* species and germination was blocked completely under darkness (Çırak *et al*. 2006, 2007). In the present study, nettle seeds could germinate effectively only in the presence of light and no pre-soaking treatment induced germination under darkness. Since light intensity in the wild is extremely high in Turkey, plants could germinate at a high level in response to exposure to increased light intensities in the laboratory. The results indicated that nettle seeds exhibit endogenous dormancy and need light to germinate effectively. It should be noted that no seed dormancy was reported for nettle by Bassett *et al*. (1977). The authors did not point out the actual germination rates their observed or whether they applied some germination promoting treatments or not. In the present study, 30% of untreated control seeds under darkness germinated, which is very high when compared to maximum seed germination level of many other species and may not be considered as "dormant". However, the same seeds germinated at 83.33% in the presence of light, approximately 3-fold higher than in darkness. Hence we preferred to describe this germination response to light as endogenous dormancy.

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 or long days (Koornneef *et al*. 2002). They are especially effective in promoting germination in case of partially dormant embryo (Cerabolini *et al*. 2004). However, GA treatments were found to be fully ineffective for inducing seed germination in the present study. This phenomenon may be attributed the fact that embryo of nettle seed is not dormant, but just needs light to start the germination process.

Seeds of many wild members of different genera have hard seed coats which restrict water absorption by the embryo resulting in limited/completely suppressed oxygen availability. 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. H_2SO_4 or NaOH) (Abdallah *et al*. 1989). Soaking the seeds in hot water or H_2SO_4 solutions were reported to be effective applications in promoting germination for many wild plant species such as *Prosopis ferox* (Baes *et al*. 2002), *Hypericum perforatum* (Çırak *et al*. 2006), *Pterolobium stellatum* (Teketay 1998) and *Dodonea viscosa* (Davis *et al*. 2004). In contrast, both hot water and, especially, H_2SO_4 treatments resulted in a significant decrease in seed germination in the present study indicating the absence of a hard seed coat in nettle seeds.

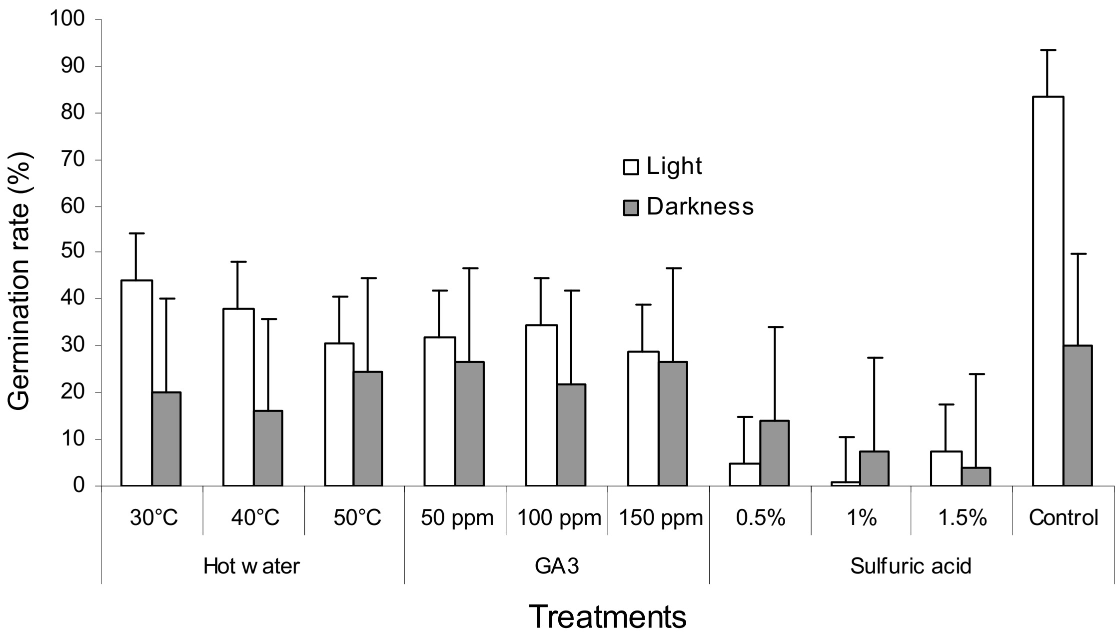

Fig. 1 The effects of different pre-soaking treatments on germination rates of nettle seeds. The seeds left to germinate under both light and darkness without soaking in chemical and hot water solutions were treated as controls. (Bars are ± SE).

CONCLUSION

Determination of germination requirements is the first step to be taken in domestication considering the fact that propagation by seed has been regarded the most suitable and effective tool in large-scale production of a given plant species. Results of the present study indicated that nettle seeds have endogenous dormancy concerning the evident light requirement for germination and no presoaking treatments are effective in eliminating this dormancy and promoting germination. Further studies on elucidating the cultivation requirements of nettle are currently underway in laboratory and experimental areas of Ondokuz Mayis University, Agricultural Faculty, Turkey.

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