Journal of Plant Biology, December 2004, 47(4) : 391-395 **SHORT COMMUNICATION**

Physical and Physiological Dormancy in Black Henbane (*Hyoscyamus niger* L.) Seeds

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Aim of this study was to investigate the nature of dormancy in black henbane (*Hyoscyamus niger*) seeds which have low germination rate under normal laboratory conditions. To do this, before placing the seeds in Petri dishes, they were soaked in 5, 10 and 15 mg/L GA; 1, 2 and 3% H_2SO_4, 15 mg/L GA + 1% H_2SO_4, 0.01 M KNO_3 solutions, tap water, 40, 50 and 60°C hot water for 30 min. The study was performed under both continuous illumination and darkness in growth chambers to evaluate the effect of light on germination rate. The results showed that H_2SO_4 and GA treatments were the most important factors affecting seed germination and their germination enhancing effects were more evident in darkness. The results also suggested that black henbane seeds exhibit double dormancy involving a hard seed coat and a partially dormant embryo and have a partial dark requirement to germinate.

Keywords: black henbane, dark requirement, double dormancy, GA, H_2SO_4

Medicinal plants have been the subjects of man's curiosity since time immemorial and almost every civilization has a history of medicinal plant use (Constable, 1990). Approximately 80% of the people in the world's developing countries rely on traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts (Constable, 1990; Vieira and Skorupa, 1993). Black henbane (*Hyoscyamus niger* L.), native to Scandinavia and southern England to the Mediterranean and northern Africa, is an annual herb of the family *Solanaceae*. This plant is a coarse, foul-smelling, and very hazardous weed with all parts being poisonous. By distillation the leaves, have long been employed as a narcoticmedicine, yield a very poisonous volatile oil, but the active principles are hyoscyamine and hyoscine (Pudersell et al., 2003). Despite this plant's weedy tendency and poisonous nature, it has great historical significance. The medicinal uses of black henbane date from remote ages; it was well known to the ancients, being particularly commended by Dioscorides (first century A.D.), who used it to procure sleep and allay pains (Hocking, 1947). Its most important use is in relief of painful spasmodic affections of the unstriped muscles, as in lead colic and irritable bladder (Mitich, 1992). Today, it has been cultivated as an ornamental plant and a crop for drug companies worldwide, especially United States, Europe and India (Pandey et al., 1999). In spite of its well-known medicinal importance, black henbane is a weed that has been studied very little. Limited information is available on this species, other than descriptions of the alkaloids it contains.

Germination is a critical stage in the life cycle of plants, and often controls population dynamics, with major practical implications (Keller and Kollmann, 1999). But, generally germination rate of weedy species, like black henbane, is very low due to seed dormancy (Radosevich et al., 1997). Over the past twenty years, dormancy has been widely studied but the regulatory principles behind changes in several types of dormancy remain unclear (Rehman and Park, 2000). Nevertheless, plant growth regulators such as GA (gibberellic acid) and IAA (indoleacetic acid) (Hilhorst and Karssen, 1992; Iglesias and Babiano, 1997); chemical substances such as KNO_3 (Kevseroğlu, 1993; Hartmann et al., 1997) and H_2SO_4 (Horowitz and Taylarson, 1985; Tomer and Maguire, 1989; 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 light and exogenously applied GA, KNO_3, H_2SO_4, hot and tap water on germination and to find an effective method for breaking seed dormancy of black henbane.

MATERIALS AND METHODS

Ten month-old seeds obtained from black henbane plants growing wild in the vicinity of Gümüşhane

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province of Turkey were used. Pre-soaking treatments used were different GA and H_2SO_4 doses, hot and tap water, and 0.01 M KNO_3. Before placing the seeds in Petri dishes, they were soaked in 5, 10 and 15 mg/L GA; 1, 2 and 3% H_2SO_4, 1% H_2SO_4 + 15 mg/L GA, and 0.01 M KNO_3, tap water, 40, 50 and 60°C hot water for 30 min. To evaluate the effect of light on germination, the study was performed under continuous illumination (1200 μmol $m^{-2}s^{-1}$ white fluorescence light) and darkness in growth chambers. Temperature was set to 20°C. For each application 3 × 100 seeds were placed in Petri dishes, and germinating seeds were counted for 14 days after treatments. Data were objected to ANOVA and differences among treatments were tested with Duncan Multiple Range Test ($P < 0.01$).

RESULTS AND DISCUSSION

The germination rates of black henbane seeds treated with different factors were shown in Table 1. According to the results of variance analysis, light and different GA and H_2SO_4 concentrations had significant effects on germination rate ($P < 0.01$). The effect of light on germination was negative. In general, germination rates were higher under darkness. As mean of light and dark conditions, GA and H_2SO_4 were found to be effective. Among pre-soaking treatments, 1% H_2SO_4 and 15 mg/L GA treatment gave the highest germination rate with 64%. This was followed by 1 and 3% H_2SO_4 treatments found in the same statistical group (26.0% and 26.5%, respectively). Besides each treatment, an interaction ($P < 0.01$) between treatments was determined to be statistically significant. In this respect, the highest germination rate was obtained from 1% H_2SO_4 and 15 mg/L GA treatment (68%) under darkness. This number was followed by 1, 2 and 3% H_2SO_4 (45, 35 and 34%) and 15 mg/L GA treatments (20%) under darkness. Germination enhancing effects of H_2SO_4 and GA observed in the present study were more evident under darkness when compared to that of light conditions.

On the contrary of light, H_2SO_4 and GA treatments, presoaking the seeds in hot and tap water and KNO_3 solution did not affect germination significantly. The treatments gave the lowest germination rates together with untreated controls under both light and darkness.

Light has been recognized since the mid-nineteenth century as a germination-controlling factor. Recent research demonstrates that light acts in both dormancy induction and release and is a mechanism that adapts plants to specific niches in the environment often interacting with other factors like temperature (Benvenuti and Macchia, 1997). Although light has no effect on germination of many crop seeds (Kacar, 1996), germination response of weeds to light is various. While some of them need light to germinate at the highest level such as some cacti species (Arechiga et al., 1997; Barrera and Nobel, 2003), *Lesquerella fendleri* (Puppala and Fowler, 2003), *Drosera anglica* (Baskin et al., 2001) and *Hypericum perforatum* (Cırak et al., 2004), some others have dark requirement for germination such as *Leptochloa chinensis* (Benvenuti et al., 2004), *Sicyos deppei* (Segovia et al., 2000), *Bromus sterilis* (Peters, 2000), *Monochoria vaginalis* (Kuo, 1999), *Nelumbo nucifera* (Ushimaru et al., 2001) and *Leymus arenarius* (Greipsson and Davy, 1994). Similarly, in the present study, black henbane seeds could germinate in the presence of light, but germination percentages were higher under darkness for all treatments except for hot and tap water treatments which were inefficient to improve germination under both light and darkness. The results suggest that black henbane seeds have a partial dark requirement to germinate to the highest level.

Seeds of many wild members of the *Leguminosae* and *Solanaceae* have hard seed coats which restrict water absorption by the embryo. Failure to imbibe limits O_2 to the embryo and leaching of inhibitors, therein effectively enforcing dormancy of the embryo.

Table 1. The effects of light and some pre-soaking treatments on germination rates of *H. niger* seeds.

	Treatments													
	Hot water (°C)			GA (mg/L)			H_2SO_4 (%)			H_2SO_4 + GA*	0.01 M KNO_3	Tap water	Control	Mean
	40	50	60	5	10	15	1	2	3					
Light	0e**	0e	0e	0e	8e	4e	7e	7e	19e	60a	2e	0e	0e	3.92
Darkness	0e	0e	0e	0e	5e	2d	45b	35c	34b	68a	2e	2e	2e	11.75
Mean	0F***	0F	0F	0F	6E	12D	26B	21C	26B	64A	2F	1F	1F	

*1% H_2SO_4+15 mg/L GA; **Values followed by different small letters in columns and rows and ***capital letters in bottom row are significantly different ($P < 0.01$) according to Duncan Multiple Range test.

For applied uses, dormancy-breaking treatments are required to provide more uniform and rapid seed germination. 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. sulfuric acid or sodium hypochlorite) (Abdallah et al., 1989). In the present study, H_2SO_4 treatments were found to be effective to induce germination, while mechanical abrasion by soaking in hot water had no effect. The results indicate the presence of physical dormancy related to hard seed coat and overcame by only acid scarifications. The high germination rates obtained with H_2SO_4 treatments agree with those obtained in other species: some legumes (Horowitz and Taylarson, 1985; Muir and Pitman, 1987; Tomer and Maguire, 1989; Teketay, 1996; Grouzis and Danthu, 2001), *Prosopis caldenia* (Pelaez et al., 1992), *Prosopis ferox* (Baes et al., 2002), *Acacia origena*, *Acacia pilispina* and *Pterolobium stellatum* (Teketay, 1998), *Erythrina brucei* and *Erythrina burana* (Teketay, 1994) and *Zamia floridana* (Dehgan and Johnson, 1983).

Seed dormancy and germination are complex adaptive traits of higher plants that are influenced by a large number of genes and environmental factors. 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. These compounds occur at relatively high concentrations in developing seeds but usually drop to a lower level in mature dormant seeds, particularly in dicotyledonous plants. 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 seeds affected by gibberellins, cell elongation is enhanced, so that the radicle can push through the endosperm, seed coat, or fruit coat that restricts its growth (Salisbury and Ross, 1992). In this study, GA increased germination rate significantly depending on used doses leading us to believe that there was a physiological dormancy related to partially dormant embryo except for physical dormancy. This phenomenon was strengthened by the fact that 1% H_2SO_4 and 15 mg/L GA treatment gave the highest germination rate. The germination enhancing effect of GA was reported from the studies carried on other species such as *Haplopappus gracilis* (Galli et al., 1975), *Sesamum indicum* (Kyauk et al., 1995), *Rumex dentatus* (Ali and Helal, 1996), *Zea mays* and *Glycine max* (Wang et al., 1996) and *Opuntia tomentosa* (Carrillo et al., 2003).

Nitrates have been commonly used for breaking of dormancy in seeds requiring light to germinate. Nitrates have increased seed germination by offsetting the light requirement on a large scale (Kacar, 1996). In a study whose main objective was to evaluate seed treatments for reducing or eliminating the light requirement of *Lesquerella fendleri* seeds, KNO_3 was reported as an effective agent for reducing light requirement and enhancing germination (Puppala and Fowler, 2003). But, in this study, KNO_3 was all generally ineffective in promoting germination and this lack of response to KNO_3 was attributed to the absence of light requirement for germination. The result is in agreement with the research of Greipsson and Davy (1994) who reported no germination response to KNO_3 treatment for *Leymus arenarius*.

Hot water treatments have reported to enhance germination of hard coated seeds by elevating water and O_2 permeability of testa (Msanga and Maghembe, 1986; Teketay, 1998; Aydın and Uzun, 2001). Likewise, Hermansen et al. (1999) reported that germination was enhanced by presoking in 44, 49 and 54°C hot water for 5-40 min in carrot seeds. But in our study, hot water treatments did not induce germination. It was, probably, due to low water temperatures which were not enough to soften hard seed coat. These data are consistent with work by Khosh-Khui and Bassiri (1976) and Masamba (1994) who did not observe significant germination response to hot water treatments in different temperate for some Acacia species and *Myrtus communis*, respectively.

Chemicals that accumulate in fruit and seed covering tissues during development and remain with the seed after harvest can be shown to act as germination inhibitors. The inhibitors have been found in the seeds of such species as *Citrus karna*, *C. jambheri* and *C. grandis* (Saipari et al., 1998), *Lycopersicon esculentum* (Cuartero and Rafael, 1998), *Purshia tridentate* (Booth and Sowa, 2001), *Bertholletia excelsa* (Karen et al., 1999) and *Hypericum perforatum* (Macchia et al., 1983). 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 (Çırak et al., 2004). Similar result was reported on *Myrtus com-*

munis by Khosh-Khui and Bassiri (1976). But, in this study, tap water treatment was fully inefficient in enhancing germination indicating that black henbane seeds may not carry chemical inhibitors that could be eliminated with the tap water treatment.

Consequently, the results from the present study showed that H_2SO_4 and GA treatments were the most important factors affecting seed germination in black henbane and their germination enhancing effects were more evident in darkness. The results suggest that black henbane seeds exhibit double dormancy involving a hard seed coat and a partially dormant embryo and have a partial dark requirement to germinate. In order to overcome these barriers, 1% H_2SO_4 and 15 mg/L GA treatment was recommended.

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