Iranian Journal of Weed Science 5 (2009) 27-38

مجله دانش علف‌های‌هرز

Mouse Barley Germination Environmental Factors Influencing Germination of Mouse Barley (*Hordeum murinum* L.) in South Khorasan Province, Iran

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(Received 13 July 2009; returned 25 Jun 2010; accepted 6 December 2010)

ABSTRACT

Mouse barley (*Hordeum murinum*), from the poaceae family, is an abundant annual grass weed in wheat fields of South Khorasan province. Determining the ecological factors of this grass weed will contribute to development of its control programs. Effects of environmental factors on germination and emergence of mouse barley was investigated in the laboratory. Although mouse barley germination was greater than 85% at salinity level of 160 mM, further increase of salinity caused a remarkable decrease in its germination, following a 3% decrease in germination at salinity level of 320mM. Increasing osmotic potential from 0 to -0.8 MPa, resulted in an 80% decrease in its germination. Mouse barley germination was not affected by the pH and in a pH range of 4 to 10 remained approximately 90%. Emergence depth test showed that the maximum emergence of mouse barley occurred for seeds placed on the soil surface (86%) and no seedlings emerged from the seeds buried at 10cm depth. High germination ability of mouse barley under diverse environmental conditions may greatly contribute to it as a problematic weed species in the wheat fields of the region.

Keywords: Salinity, osmotic potential, emergence depth, acidity.

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INTRODUCTION

Mouse barley, also referred to as barley grass or wild barley, is a winter annual grass that grows in non cropland and croplands of Southern Khorasan. Its native range extends from central Europe, south to northern Africa and east to western Asia and the Caucasus (USDA, ARS 2005). Mouse barley grows well in warm and dry areas, while cold and moist conditions hinder its growth (Davison, 1977). It propagates by seeds which are produced in large number (Halloran & Pennell 1981). It is a successful invader species, in disturbed and, soil high in nutrient levels and nitrogen (Dean, 1990). Mouse barley establishes easily on land subjected to regular grazing and becomes dominant with increasing intensity of grazing (Groves *et al*., 2003). After flowering in the spring, the grass matures rapidly to produce a large number of seeds. The majority of mouse barley seeds remain dormant during the summer till autumn. Seed dormancy is an important mean of avoiding the catastrophes that weeds encounter (Baker, 1974) and the ability of a seed to persist in the soil is favorable in disturbed habitats where synchronous germination could result in the mortality of all plants (Cohen, 1966; Westoby, 1981). The high level of dormancy is usually a general characteristic of annual weeds that results in large, persistent seed banks (Anderson, 1990). An appropriate seed dormancy strategy will enable winter weed species to germinate under favorable conditions of late autumn and winter and also prevents germination after summer rains, which are invariably followed by a period of drought (Dunbabin & Cocks, 1999).

The success of mouse barley as an invader specie throughout the world has been attributed to its early germination and rapid growth rate, seed dormancy, high seed production and efficient dispersal mechanisms. It has a short life cycle and 19 to 29 seeds are produced per head (Dean, 1990).

Various environmental factors, such as temperature, osmotic potential, pH, light quality, management practices and seed location in the soil seed bank as well as soil texture have been known to influence weed seed germination and emergence (Norsworthy & Oliveira, 2006). To understand why mouse barley is so troublesome, it is important to gain a better understanding of how its seeds germinate in response to different environmental factors such as light, temperature, osmotic potential, pH, and burial depth (Chachalis & Reddy, 2000; Chauhan & Johnson, 2008). A better understanding of mouse barley germination could aid strategies to manage this weed. Develop offing models helps to predict germination and emergence or the influence of tillage and burial on the two mentioned processes. Longevity is influenced by several factors, including the physiological status of seeds, the chemical and physical environment where seeds reside, and the position of seeds relative to the soil surface (Baskin & Baskin, 1998).

Although mouse barley is a problematic weed species in Southern Khorasan cereal

farms (Tareghian, 2002), little information on effects of environmental factors on its germination biology exists. Knowledge about mouse barley germination would help estimating the potential of its spread to new croplands. Therefore, this study was conducted in order to understand the influence of different environmental factors on the seed germination and seedling emergence of mouse barley.

MATERIALS AND METHODS

Site and Seed Description

Mature mouse barley seeds were collected in June 2007, from several wheat fields at the Amirabaad Campus, Birjand, in Southern Khorasan (latitude = 32º 56´N, longitude = 59º 13´E and 1480 m altitude). Seeds from 500 plants were separated from the inflorescence and pooled to obtain seed samples. 3 months after maturity, the seeds were stored in paper bags at a constant temperature (4 ± 1C). An 1,000-seed weight of mouse barley was 4.2 ± 0.01 g.

General Protocol for Germination Tests

Four 25-seeds of mouse barley were placed in 9-cm petri dishes lined with 2 discs of filter paper, moistened with either 5mL deionized water or treatment solution when required. The petri dishes were sealed with parafilm to minimize evaporation and placed directly in the germinator or wrapped in two layers of aluminum foil to exclude light prior to placing them in the germinator. Germination tests were conducted for 14 days at a day/night temperature of 25 ˚C/15˚C (12h/12h). Seeds were considered to have germinated when the radicle emerged. The number of germinated seeds was counted at the end of the germination test. In preliminary testing, freshly harvested seeds placed in petri dishes did not germinate under normal laboratory conditions.

Temperature and Light

Experiments were conducted to determine the effects of various fluctuating day/night temperatures (15/6, 20/10, 25/15, 30/15 & 35/20 ˚C) on seed germination (3 months after maturity) under light/dark and continuous dark regimes. This temperature regime was chosen to simulate the late summer and autumn temperatures in Southern Khorasan.

Salinity

Sodium chloride solutions of 0, 10, 20, 40, 80, 160, 320 and 640 mM were prepared in deionized water. These salinity concentrations were chosen to cover variations in the level of salinity in South Khorasan soils. Petri dishes were incubated as described in the general protocol under light/dark regime.

Solution Osmotic Potential

Mouse barley seeds were germinated in the light/dark in aqueous solutions of polyethylene glycol 6000 with osmotic potentials of 0, -0.1, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa, prepared by dissolving appropriate amounts of PEG 6000[2] in deionized water (Michel, 1983).

PH

The effect of pH on germination was studied using buffer solutions of pH = 4 to 10 according to the method described by Chachalis & Reddy (2000). A 2 mM potassium hydrogen phthalate buffer solution was adjusted to pH 4 with 1 N HCl. A 2 mM solution of MES [2-(*N*-morpholino) ethanesulfonic acid] was

adjusted to pH 5 and pH of 6 with a 1 N NaOH. A 2-mM solution of HEPES [*N*-(2-hydroxymethyl) piperazine- *N*-(2-ethanesulfonic acid)] was adjusted to pH 7 and pH 8 with 1 N NaOH. Buffer solutions of pH 9 and pH 10 were prepared with 2-mM tricine [*N*-Tris(hydroxymethyl methylglycine] and adjusted with 1 N NaOH. Petri dishes were incubated at 25/15 ˚C day/night temperature as described in the general protocol.

Emergence Depth

The effect of different planting depths on seedling emergence of mouse barley was investigated in a growth chamber. Seeds were placed at five different depths (0 cm or surface, 2.5, 5, 7.5, and 10 cm) in 15-cm-diam plastic pots. Control pots, where seeds were not added, indicated that there was no background seed bank of mouse barley in the study soil. Moist soil was placed over sown seeds to the appropriate depth and gently compacted. For each burial depth, four pots (replicates), with 50 seeds per pot were set up (total of 20 pots). Soil used for this experiment was a loamy soil comprised of 43% sand, 32% silt and 25% clay with 0.44% total organic matter and a pH of 7.4. The temperature of the growth cabinet was set to 25/15 ˚C (day/night). The photoperiod was set to 12 hours with fluorescent lamps used to produce a light intensity of 140 µmol m^{-2} s^{-1}. The pots were watered as needed to maintain adequate soil moisture. Seedlings were counted as they emerged through the soil and the experiment was run till 45 days after burial. Mean emergence time (*MET*) was calculated using the following formula

$$MET = \sum (n \times g) / N$$

in which, *n* is the number of seedlings emerging per day, *g* is the number of days needed for emergence, and *N* is the total number of emerged seeds. At the end of the experiment, seeds buried at 10 cm depth were recovered to determine the fate of non germinated seeds. The soil was filtered using a 1 mm mesh metal sieve to recover intact (dormant) seeds as well as seedlings that were rotting due to failure of emergence after germination. This procedure made it possible to distinguish between seeds that remained dormant and germinated seeds that failed to emerge due to excessive depth of burial.

Data Analysis

All experiments were carried out twice (except the emergence depth study) as a completely randomized design with four replicates per treatment. The data of the experiments were pooled for analysis, as there was no time-by-treatment interaction. A functional three parameter logistic model (Chauhan *et al.,* 2006a) as expressed below:

$$G\ (\%) = G_{max}/[1 + (x/x_{50})^{Grate}] \qquad [1]$$

was fitted to the germination values (%) obtained at different concentrations of NaCl or osmotic potential using SigmaPlot[3] (version 11.0). In this equation, *G* represents the total germination (%) at NaCl concentration or osmotic potential *x*, G_{max} is the maximum germination (%), x_{50} is the NaCl concentration or osmotic potential for 50% inhibition of the maximum germination, and G_{rate} indicates the slope. The seedling emergence values

(%) obtained at different burial depths were fitted to a sigmoidal decay curve (Norsworthy and Oliveira, 2006) in the form of:

$$E\ (\%) = E_{max} / (1 + \exp(-(x - x_{50}) / E_{rate}))$$

where E represents the seedling emergence (%) at burial depth x, E_{max} is the maximum seedling emergence, x_{50} represents the depth at which emergence is reduced by 50%, and E_{rate} indicates the slope. Transformation of data did not improve homogeneity; therefore, ANOVA and regression analysis were performed on non-transformed percentage of germination (Genstat[4], version 9.2). Means were separated using either LSD test at P = 0.05 or standard error bars.

RESULTS AND DISCUSSION

Temperature and Light

Although light and fluctuating temperatures can be important in stimulating germination of many agricultural weeds, the impact of temperature and light on mouse barley germination was insignificant (data not shown). There was no interaction between temperature and light. Mouse barley showed high germination (>90%) at all tested temperatures under both light/dark and continuous dark regimes. Results showed that mouse barley could germinate over a wide range of temperature regimes from 15/6 to 35/20 ˚C alternating day/night temperatures. Studies in Australia similarly showed that germination of mouse barley occurred at constant temperatures ranging from 7 to 32 ˚C, with optimum temperatures between 18 and 24 ˚C (Groves *et al*., 2003). The effect of large diurnal temperature swings on the germination was insignificant (Cocks & Donald, 1973, Piggin *et al*., 1973, Popay, 1981). The ability of mouse barley to germinate over a wide range of temperatures supports the extended period of its emergence in the field throughout most autumn and winter months in south Khorasan.

This study also indicated that mouse barley does not require light for germination (non-photoblastic). Exposure to light breaks dormancy in many weed species, but there are species in which light has no effect or may even inhibit germination. Grime and Jarvis (1976) also found that mouse barley seeds maintained under high or low light intensity or in darkness, germinated completely in all conditions.

Baskin and Baskin (1998) summarized that among 54 grass species, germination of 28 was promoted by light, 13 were unaffected by light or dark conditions, and 13 were inhibited by light. Milberg *et al*., (1996) tested 44 species, mostly agricultural weeds, and found that germination improved in 24 species after a 5-s exposure to light. In the remaining 20 species, there were no effects. Buhler, (1997) found that germination percentage of annual grasses like *Echinochloa crus-galli* L., *Alopecurus myosuroides* Huds, and *Setaria glauca* L. were similar under presence or absence of light. Seed germination of *Atriplex stocksii* Boiss. (Khan & Rizvi, 1994) and *Suaeda fruticosa* Forssk.(Khan & Ungar, 1998) were not inhibited by the absence of light. Germination of *Aegilops cylindrica* host has also been shown to be insensitive to

light and dark conditions (Baskin & Baskin, 1998). In situations where light promotes germination, it has been associated with small, rather than large seed masses (Milberg *et al*., 2000; Schutz *et al*., 2002).

Salinity

The three-parameter logistic model {G (%) = 98.6/[1 + (x/207.9)$^{8.6}$], r^2 = 0.99} provided a satisfactory fit for the data of mouse barley seed germination obtained at different concentrations of NaCl (Figure 1). Germination was greater than 95% up to 80 mM NaCl and also high at 160 mM NaCl (> 85%). Increasing NaCl concentration to 320 mM sharply decreased mouse barley germination (<3%). The parameter x_{50} (Equation 1) that represents the NaCl concentration for 50% inhibition of the maximum germination, was 207.9 mM NaCl, indicating a moderately salt tolerance in this species at germination stage. The results show that mouse barley would be able to germinate even at saline soil types which are common in Southern Khorasan (Forooghifar & Shahidi, 1999).

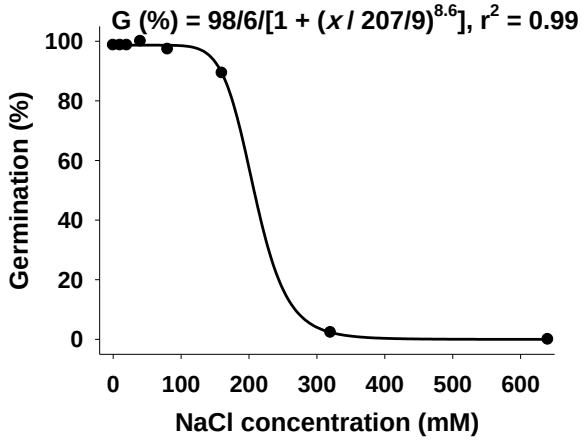

Figure 1. Effect of NaCl concentration on germination of mouse barley seed incubated at 25/15 ˚C day/night temperature with a 12-hour photoperiod for 2 weeks.

Solution Osmotic Potential

Increased solution osmotic potentials reduced mouse barley germination (Figure 2). Increasing osmotic potential from 0 to –0.6 MPa caused an 80% reduction in mouse barley germination.

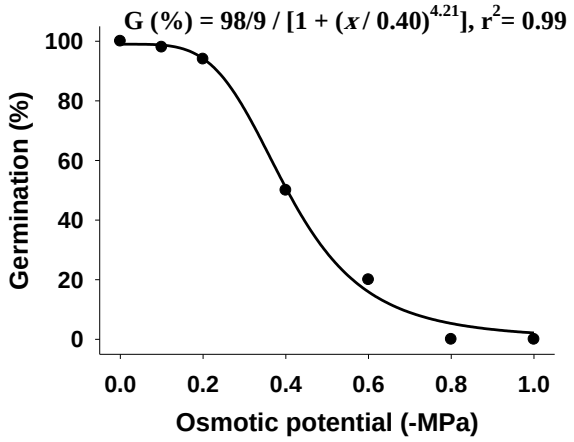

Figure 2. Effect of osmotic potential on germination of mouse barley seed incubated at 25/15 ˚C day/night temperature with a 12-hour photoperiod for 2 weeks.

The three-parameter logistic model {G (%) = 98.9/[1 + $(x/0.40)^{4.2}$], r^2 = 0.99} provided a satisfactory fit for the data of mouse barley seed germination obtained at different osmotic potentials. As osmotic potential decreased from 0 to -0.6 MPa, mouse barley seed germination decreased from 98 to 20%. No germination occurred when the osmotic potential was -0.8 and -1.0 MPa.

The osmotic potential required for 50% inhibition of maximum germination (x_{50}), determined from the fitted model (Equation 1) was -0.40 MPa. Although at lower incidence, mouse barley can still germinate under moderate water-stress conditions. Germination over a broad range of osmotic potentials indicates that mouse barley could pose a weed threat under both adequate and moderate moisture-stress soil conditions.

PH

Mouse barley seeds germinated greater than 90% over a pH range from 4 to 10 (data not shown) while most soils of South Khorasan fall in the neutral to alkaline range (Forooghifar & Shahidi, 1999). These results suggest that pH should not be a limiting factor for germination of this weed species in most soils. Similar to mouse barley, other weed species, including annual sowthistle (*Sonchus oleraceus* L. SONOL) (Chauhan *et al*., 2006a) horseweed (*Conyza canadensis* L. Cronq. ERICA) (Nandula *et al*., 2006) texasweed [*Caperonia palustris* (L.) St. Hil.] (Koger *et al*., 2004), and giant sensitiveplant (*Mimosa invisa* Mart. ex Colla MIMIN) (Chauhan & Johnson, 2008), germinated in a wide range of pH. High germination of mouse barley over a broad range of pH has implications for a widespread distribution and infestation in South Khorasan.

Emergence Depth

Depth of burial strongly influences seedling emergence. Germination was noted the 9th day after establishing the experiment for seeds placed on the soil surface (27%) and for seeds sown at 2.5 cm (21%) (data not shown). Mouse barley emergence was influenced by seeding depth ($P < 0.001$). Increasing the seeding

depth decreased emergence percentage and also delayed the emergence time (Figure 3). The greatest mouse barley emergence occurred for seeds placed on the soil surface (86%) and no seedlings emerged from burial depth 10 cm. The sigmoidal decline model {*E (%)* = 84.2 / (1 + *exp* (-(x-4.7)/-1.2)), r^2 = 0.97} was best fitted to the data of mouse barley emergence in relation to seeding depth (Figure 4). According to the model, the seeding depth that decreased mouse barley emergence by 50% was 4.5 cm. Popay and Sanders (1975) also reported that mouse barley seedlings from seed sown on the soil surface emerged well but the seed was slow in germination. They also found that seed sown at 2 mm below the surface or at 2.5 cm deep emerged well but with seed sown at 5, 7.5, and 10 cm emergence was gradually declined.

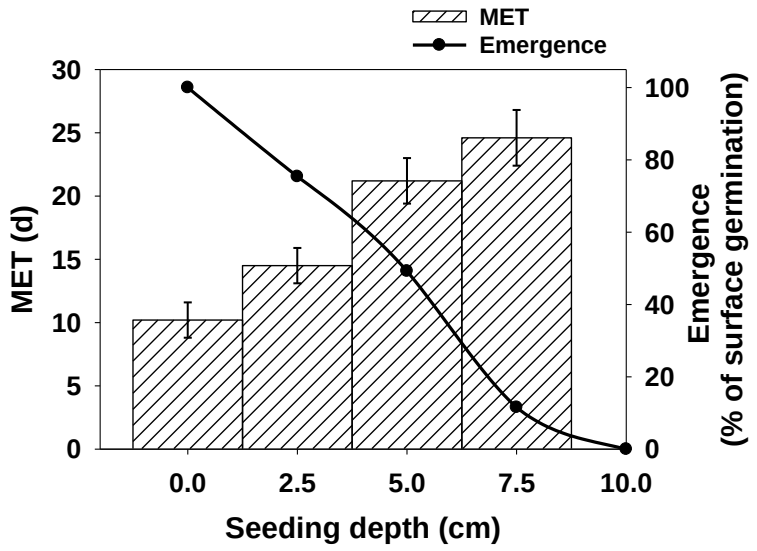

Figure 3. Effect of seeding depth on seedling emergence (as percentage of soil surface germination) and mean emergence time (MET) of mouse barley seedlings. Vertical bars represent standard errors.

Emergence of weeds is affected by the amount of food reserves in seeds and the depth of seed burial in the soil. Decreased emergence due to increased planting depth has been reported in several weed species, including horseweed (*Conyza canadensis* L.) (Nandula *et al*., 2006), sicklepod (*Senna obtusifolia* L.) (Norsworthy & Oliveira, 2006), and African mustard (*Brassica tournefortii* L.) (Chauhan *et al*., 2006b). Roberts, (1986) found that mouse barley seeds sown in a 7.5 cm layer of soil in cylinders sunk in the field, emerged mainly in the year of sowing with less than 1% of seedlings emerging in year 2 and none thereafter. Recovery of nongerminated seeds showed that failure to emerge was almost entirely the result of fatal germination (95%), rather than re-induction of dormancy. Similarly (Popay & Sanders, 1975) found that the mouse barley seeds sown deeper than 10 cm did germinate but failed to emerge. Therefore, it is thought to be unlikely that mouse barley would build up a large seedbank in the soil. As mouse barley does not form long seed bank duration (Popay & Sanders, 1975), burial of seed by deep summer

tillage may prevent germination in the following autumn. Emergence from depth of 7.5 cm could allow mouse barley to escape control with pre-emergence herbicides.

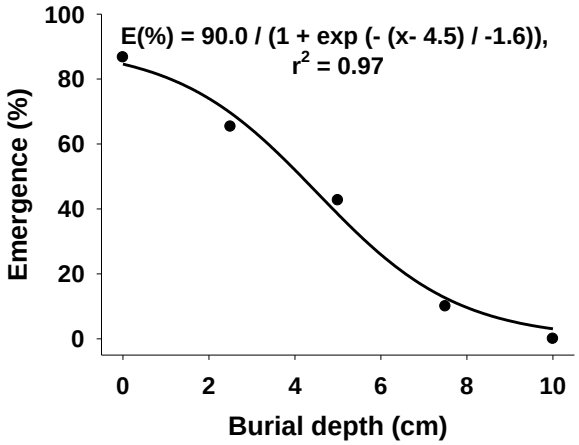

Figure 4. Effect of seed burial depth on seedling emergence (%) of mouse barley in a growth chamber maintained at 25/15 °C (day/night) temperature.

Our data suggest that mouse barley is able to germinate under a broad range of environmental conditions. Light, temperature and solution pH levels used in this study had little impact on mouse barley germination. On the contrary, water stress, salinity and depth of burial affected seed germination of this weed. Deep tillage may prevent emergence of this troublesome weed species and can be adopted as an effective tool for its control as it showed fatal germination at deep burial depths.

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چکیده

جوموشی، علف هرزی یکساله از خانوادهٔ گندمیان، در مزارع گندم استان خراسان جنوبی به وفور یافت شده و کنترل آن در برخی مزارع بسیار مشکل است. شناخت اکولوژی جوانه‌زنی این علف‌هرز به توسعهٔ برنامه‌های کنترل آن کمک خواهد کرد. اثرات عوامل محیطی مختلف بر جوانه‌زنی و سبز شدن جوموشی در آزمایشگاه مورد تحقیق قرار گرفت. اگر چه در شوری معادل ۱۶۰ میلی‌مولار کلرور سدیم، میزان جوانه‌زنی بیش از ۸۵ درصد بود، افزایش سطح شوری از آن به بعد من‌جر به کاهش قابل ملاحظه ای در جوانه زنی گردید، به طوری که در شوری ۳۲۰ میلی‌مولار، میزان جوانه‌زنی به ۳ درصد کاهش یافت. افزایش پتانسیل اسمزی از صفر به ۰/۸- مگاپاسکال، منجر به کاهش جوانه زنی به میزان ۸۰ درصد گردید. جوانه‌زنی جوموشی به وسیلهٔ pH تحت تأثیر قرار نگرفت و در دامنهٔ pH از ۴ تا ۱۰ در حدود ۹۰ درصد باقی ماند. آزمایش عمق سبز شدن نشان داد که حداکثر سبز شدن این علف - هرز از بذور سطح خاک حاصل شد (۸۶ درصد) و هیچ گیاهچه ای از بذور دفن شده در عمق ۱۰ سانتیمتری خاک سبز نشد. قابلیت جوانه‌زنی بالای جوموشی تحت شرایط م‌حیطی متفاوت ممکن است تا حد زیادی در موفقیت این علف هرز به عنوان یک گونهٔ مشکل ساز در مزارع گندم منطقه سهیم باشد.

کلمات کلیدی: شوری، پتانسیل اسمزی، عمق رویش، اسیدیتی