INFLUENCE OF ENVIRONMENTAL FACTORS ON SEED GERMINATION AND SEEDLING EMERGENCE OF YELLOW SWEET CLOVER (*Melilotus officinalis*)[1]

Influência de Fatores Ambientais na Germinação e Emergência das Plântulas de Trevo Doce Amarelo ***(Melilotus officinalis)***

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ABSTRACT - Laboratory and greenhouse experiments were conducted to determine the effects of drought and salinity stress, temperature, pH and planting depth on yellow sweet clover (*Melilotus officinalis*) germination and emergence. Base, optimum and ceiling germination temperatures were estimated as 0, 18.47 and 34.60 °C, respectively. Seed germination was sensitive to drought stress and completely inhibited at a potential of -1 MPa, but it was tolerant to salinity. Salinity stress up to 90 mM had no effect over the *M. officinalis* seed germination, but the germination decreased by increasing the salt concentration. The drought and salinity required for 50% inhibition of maximum germination were 207 mM and -0.49 MPa, respectively. High percentage of seed germination (>92%) was observed at pH = 5-6 and decreased to 80% at acidic medium (pH 4) and to 42% at alkaline medium (pH 9) pH. Maximum seedling emergence occurred when the seeds were placed at 2 cm depth and decreased when increasing the depth of planting; no seed emerged from depths of 10 cm.

Keywords: germination, pH, salinity stress, soil depth, temperature, water stress.

*RESUMO - Experimentos de laboratório e de casa-de-vegetação foram conduzidos para determinar os efeitos dos estresses de seca, salinidade, temperatura, pH e a profundidade de plantio sobre a germinação e a emergência do trevo amarelo doce (**Melilotus officinalis**). Temperaturas base, ótima e teto para germinação de **M. officinalis** foram estimados em 0, 18 e 34 °C, respectivamente. A germinação das sementes mostrou-se sensível ao estresse hídrico e foi totalmente inibida nos potenciais de -1 MPa. A germinação de **M. officinalis** foi tolerante à salinidade. Estresse salino até 90 mM não tiveram efeito sobre a germinação de sementes de **M. officinalis**, mas a germinação decresceu com o aumento da concentração de sal. A seca e a salinidade necessária para inibição de 50% de germinação máxima foi de 207 mm e -0,49 MPa, respectivamente. Alta porcentagem de germinação (>92%) foi observada em pH = 5-6 e desceu para 80% em condições ácidas (pH 4) e para 42% sob condições alcalinas (pH 9). Emergência máxima ocorreu quando as sementes foram posicionadas na profundidade de 2 cm e diminuiu com o aumento da profundidade de plantio. Nenhuma semente emergiu quando a profundidade de semeadura foi de 10 cm.*

Palavras-chave: germinação; pH; estresses abióticos, profundidade do solo, temperatura, estresse hídrico.

INTRODUCTION

Yellow sweet clover (*Melilotus officinalis*) is known as field melilot, ribbed melilot and yellow melilot, which is a dicotyledonous summer annual or biennial herb and a member of family *Fabaceae*. Pods are pale brown and usually have one seed, rarely two, and each plant produces 100,000 seeds that are purple specks (Turkington et al., 1978).

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The seeds remain viable in the soil seed bank for at least 20 yrs (Smit & Gorz, 1965). The species is native from Eurasia and naturalized from Ireland throughout Europe and Asia to Western China (Turkington et al., 1978). In recent years, *M. officinalis* has become a problematic weed in canola and wheat fields in almost all parts of Golestan province, in Iran (Yones-Abadi, 2003). This plant substantially reduces the yield of several major crops (Turkington et al., 1978), but its interference with the canola harvesting and the reduction of the crop seed quality is probably the most important economic consequence of the presence of this weed on the fields.

Germination and emergence are the two most important stages in the life cycle of plants that determine the efficient use of the nutrients and water resources available to plants (Gan et al., 1996) and can compete for an ecological niche (Forcella et al., 2000). Environmental factors such as temperature, light, pH, and soil moisture are known to affect seed germination (Martins et al., 2000; Canossa et al., 2008; Ikeda et al., 2008; Rizzardi et al., 2009). Temperature plays a major role in determining the periodicity of seed germination and the distribution of species (Guan et al., 2009). Germination rate usually increases linearly with temperature, at least within a well-defined range, and declines sharply at higher temperatures (Alvarado & Bradford, 2002). Other major factors that affect seed germination are water potential and salinity. Researches showed that osmotic and salt stress can delay, reduce or prevent germination (Zhou et al., 2005). In addition to these factors, germination is also affected by pH and planting depth (Lu et al., 2006; Norsworthy & Oliveira, 2006)

A better understanding of germination and emergence of *M. officinalis* in relation to environmental variables may explain why this plant is a common weed of some crops, specially canola. Also, detailed knowledge about the environmental conditions required for weed seed germination and seedling emergence in soil is an important prerequisite for the development of an integrated and environmentally-safe strategy of weed management. The ability to predict seed germination and emergence in response to environmental conditions is essential in order to allow better timing of mechanical, biological, and other management strategies (Ghorbani et al., 1999).

To date, there is no published information on seed germination and seedling emergence of *M. officinalis*. Therefore, the purpose of this research was to study the effects of temperature, osmotic stress and salt stress, pH, and planting depth on yellow sweet clover seed germination and seedling emergence.

MATERIALS AND METHODS

Seed samples

M. officinalis pods were collected from plants growing in a canola field in Golestan province of Iran in late May 2004. The seeds were removed from the pods and kept on a refrigerator at 10 ± 2 °C until used. To break seed dormancy, the seeds were soaked in concentrated sulfuric acid (98%) for 20 min and then rinsed with distilled water to get the 99% germination (Ahmadi & Ghaderi, 2005).

Effect of temperature

Seed germination was tested on 3 replicates of 50 seeds in moist paper towels in the incubators at constant temperatures of 0, 5, 10, 20, 25, 30, 32 and 35 °C in darkness. Seeds were observed twice daily and considered germinated when the radicle was approximately 2 mm long or more. Water was added as required. The distilled water used for replenishment of moisture was kept in an incubator to ensure a water temperature consistent with the incubator.

Time estimates taken for cumulative germination to reach 50% of its maximum at each replicate (D_{50}) were interpolated from the germination progress curve versus time. Germination rate (R_{50} h^{-1}) was then calculated as (Soltani et al., 2002):

$$R_{50}=1/D_{50} \qquad \text{Equation (1)}$$

Effect of salt stress

To investigate the effect of salt stress on seed germination of yellow sweet clover, three replicates of 50 seeds were incubated in moist paper towels with sodium chloride solutions of 0, 45, 90, 135, 180, 225, 270 and 315 mM. The seeds were incubated at 18 °C in darkness.

Effect of osmotic stress

To evaluate the effect of osmotic stress on seed germination of yellow sweet clover, three replicates of 50 seeds were incubated in moist paper towels containing solutions with osmotic potentials of 0, -0.2, -0.4, -0.6, -0.8, -1.0 and -1.2 MPa, prepared by dissolving polyethylene glycol 8000 in distilled water, as described by Michel (1983). The seeds then were incubated at 18 °C in darkness.

Effect of pH

To determine the effect of pH on seed germination of yellow sweet clover, three replicates of 50 seeds were incubated in moist paper towels and buffer solution with pH values of 4, 5, 6, 7, 8 and 9. Buffer solutions were prepared using potassium hydrogen phthalate in combination with either 0.1 M HCl or 0.1 M NaOH to obtain solution pH levels of 3, 4, 5, and 6. A 25 mM sodium tetraborate decahydrate solution was used in combination with control either of 0.1 M HCl or 0.1 M NaOH to obtain solution pH levels of 7, 8, and 9 (Shaw et al., 1991). Distilled water (pH=6.2) was used as control. The seeds were incubated at 18 °C in darkness.

Effect of planting depth

Thirty seeds of yellow sweet clover were placed on soil in plastic pots with 15 cm-diameter and 15 cm-height at depths of 0, 2, 4, 6, 8, 10 and 12 cm. The soil used was silty clay loam with a pH of 6.8 and 1.21% organic matter. For all treatments, the bottom and uppermost layer of soil were leveled and then pressured before and after seed placement to standardize depths and improve seed-soil contact. Seedling emergence was monitored daily for 10 days. Seedlings were counted at the appearance of the cotyledon and removed by cutting them at the soil surface.

Statistical analyses

All experiments were conducted with the use of a completely randomized design with three replicates. Prior to analysis, the germination percentage was transformed by arcsine square.

The Segmented model (Equation 2) was used to quantify the response of germination rate to temperature and to estimate cardinal temperatures (Soltani et al., 2006).

$$f(T) = (T - T_b) / (T_o - T_b) \quad \text{if} \quad T_b < T \leq T_o \qquad \text{Equation (2)}$$

$$f(T) = (T_c - T) / (T_c - T_o) \quad \text{if} \quad T_o < T < T_c$$

$$f(T) = 0 \quad \text{if} \quad T \leq T_b \text{ or } T \geq T_c$$

where, T is the temperature, and T_b, T_o and T_c are the base, optimum and ceiling temperatures, respectively.

pH data was subjected to a binomial model, whereas planting depth data was fitted to a cubic model. Salt and osmotic stress data showed a sigmoid trend and a three-parameter logistic model was fitted to data (Equation 3).

$$G(\%)=Gmax/(1+(x/x_{50})\text{^}\mathrm{Grate}) \qquad \text{Equation (3)}$$

where, G is the total germination (%) at concentration x, $Gmax$ is the maximum germination (%), x_{50} is the NaCl or osmotic potential required for 50% inhibition of the maximum germination and *Grate* indicates the slope of the curve in x_{50}. Statistical Analysis System (SAS) was used for analyzing data (SAS, 1989).

RESULTS AND DISCUSSION

Effect of temperature on germination

Maximum germination attained was 80-97% and occurred between 15 and 30 °C (Figure 1A). A marked decrease in germination occurred when temperature was outside this range and germination reached zero at 0 and 35 °C.

The influence of the temperature on the germination rate was described by a Segmented model (Figure 1B). Crops have three cardinal temperatures which are the base, ceiling, and optimal temperatures. Germination rate increases from the base to optimum temperature and then decreases to

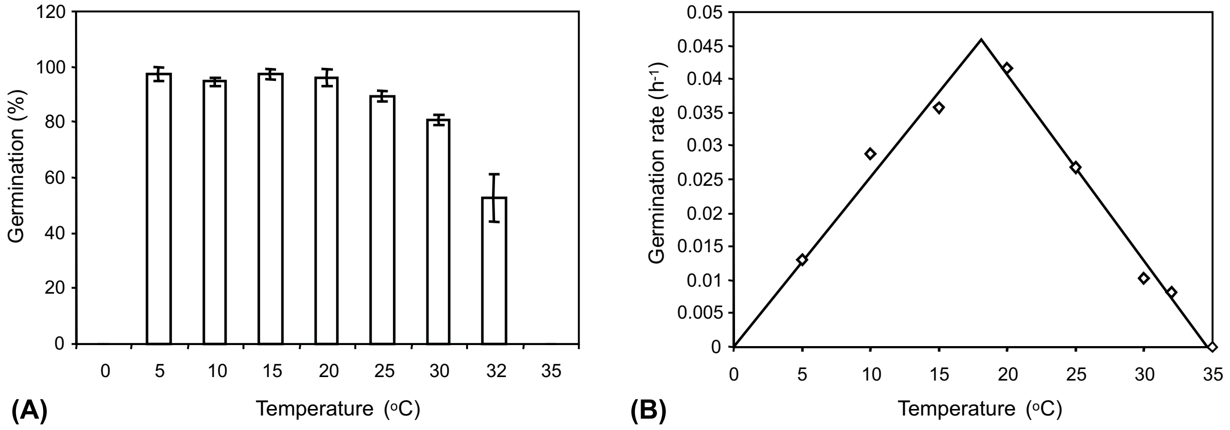

Figure 1 - Effect of constant temperature on (a) germination percentage and (b) germination rate of yellow sweet clover.

a ceiling temperature (Alvarado & Bradford, 2002). Base, optimum and ceiling temperatures were estimated as 0, 18.47 and 34.60 °C, respectively (Figure 1B). Similar type of results were observed for different plants (Vidal et al., 2007; Wu et al., 2007).

Effect of salt and osmotic stress on germination

Seed germination did not decrease significantly from 0 to 100 mM, but beyond this point declined with increasing salinity following a sigmoid trend and was completely inhibited at 300 mM salinity (Figure 2). The concentration required for 50% inhibition was 100 mM NaCl. These results suggest that even at high soil salinity, yellow sweet clover seeds may germinate. Similar results have been reported for *Mimosa pudica* (Souza Filho et al., 2001) and *Beckmannia syzigachne* (Rao et al., 2008). Salinity might negatively affect some important physiological processes in plants. Additionally, sodium ions can alter soil structure and fertility by replacing calcium and magnesium in anion exchange process, and this leads to nutrient and water stress (Rao et al., 2008).

M. officinalis seed germination was obviously affected by increasing water stress. Germination percentage decreased from 92 to 5% as the osmotic potential decreased from 0 to -0.10 MPa, and was completely inhibited at osmotic potential of -1.2 MPa (Figure 3). The osmotic potential required for 50% inhibition

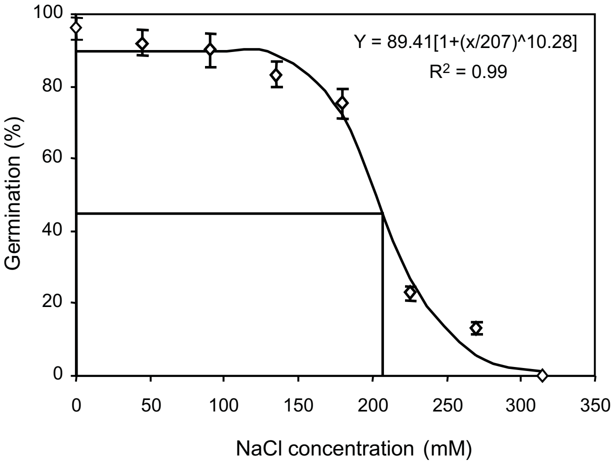

Figure 2 - Effect of NaCl concentration on germination of yellow sweet clover.

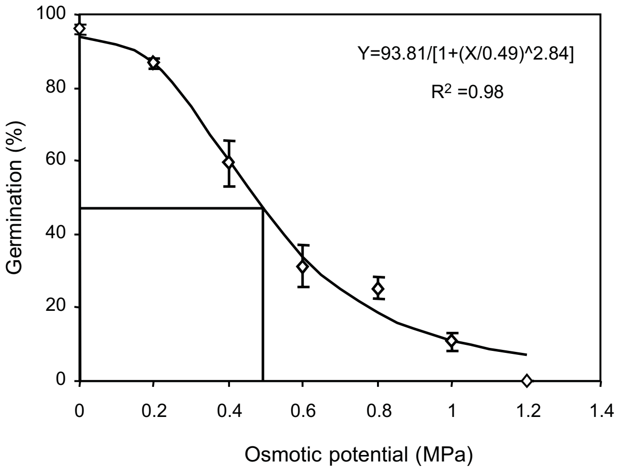

Figure 3 - Effect of osmotic potential on germination of yellow sweet clover.

of maximum germination was -0.49 MPa. Germination decreased sharply as the osmotic potential decreased beyond -0.2 MPa, suggesting that yellow sweet clover favors a moist environment for germination. These findings indicate that yellow sweet clover is rather sensitive to low water potential. Our data were similar to those reported for *Urena lobata* (Wang et al., 2009). In contrast, some other weed species were tolerant to low osmotic potential, such as *Solanum sarrachoides* (Zhou et al., 2005). According to Bradford (1990), seed germination is a process of growth of a previously quiescent or dormant seed starting with the imbibition of water. Seed imbibition rate and germination percentage decrease as the surrounding water potential decreases. *M. officinalis* seed germination was tolerant to salt stress but sensitive to low osmotic potentials, suggesting that yellow sweet clover is dependent on precipitation or irrigation for germination under field conditions.

Effect of pH on germination

The seed germination has occurred in a range of pH between 4 and 9, but the maximum germination (99%) was observed at pH=6 (Figure 4). Germination decreased to 70 and 45% at pH 4 and 9, respectively. Based on these results, yellow sweet clover germinated most readily near neutral pH, but can germinate under both acidic and alkaline soils and the pH of soil could not be a limiting factor for its germination. Germination over a broad range of pH has been reported in other weed species such as *Mimosa pudica* and *Ipomoea asarifolia* (Souza Filho et al., 2001), and *Lolium rigidum* (Chauhan et al., 2006). In contrast, poor germination occurred at extreme pH values in *Dactyloctenium aegyptium* (Burke et al., 2003), and *Eupatorium adenophorum* (Lu et al., 2006).

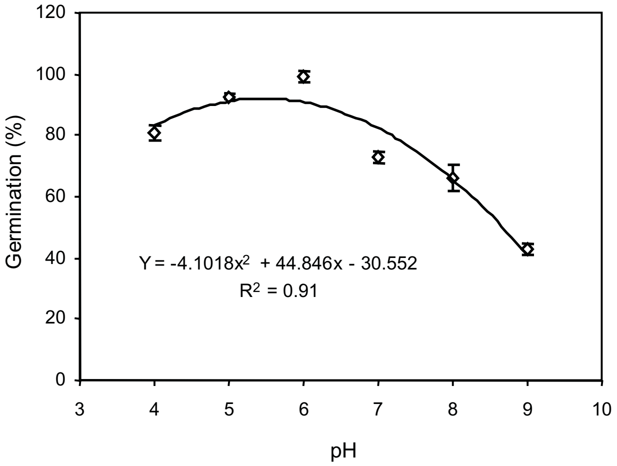

Figure 4 - Effect of pH on germination of yellow sweet clover.

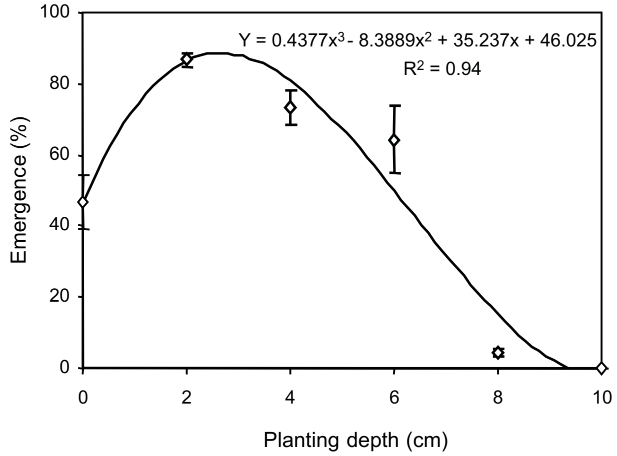

Figure 5 - Effect of planting depth on emergence of yellow sweet clover.

Effect of planting depth on emergence

Seedling emergence of yellow sweet clover was influenced by planting depth (Figure 5). Maximum emergence (87%) occurred at a planting depth of 2 cm. Seeds placed on the soil surface had low emergence compared with seeds placed at 2 cm. Limited soil to seed contact and declined water availability are some environmental conditions that may limit germination of seeds on the soil surface (Clewis et al., 2007). Seedling emergence slightly decreased as planting depth increased from 2 to 6 cm but decreased sharply at depth > 6 cm. Only 4% of seedlings emerged at depth of 8 cm, whereas no seedlings emerged from 10 cm.

Decreasing in seedling emergence due to increasing planting depth has been reported in several weed species (Lu et al., 2006; Norsworthy & Oliveira 2006; Vidal et al., 2007) and is related to light and seed size. Benvenuti (1995) reported that very little light is transmitted below a 4 mm depth in all soil types.

This research suggests that light is not a requirement for *M. officinalis* seed germination because 64% of seeds germinated at a depth of 6 cm. Also, lower seedling emergence of seeds at deep depths may be linked to limited seed reserves (Mennan & Ngouajio, 2006). Larger seeds often have greater reserves and are able to emerge from greater depths (Baskin & Baskin, 1998). *Sicyos angulatus* germinated from depth of 16 cm (Mann et al., 1981). One application we gave for the fact that *M. officinalis* seeds are able to germinate at a depth of 6 cm is that most of yellow sweet clover seeds could be sensitive to soil applied herbicides (Wang et al., 2009). Also, a possible management option for farmers may be a deep tillage operation that will bury the seeds below 8 cm (maximum depths of emergence) because seeds of yellow sweet clover cannot emerge from depths deeper than 8 cm.

The results indicate that *M. officinalis* is able to germinate and emerge in a variety of environmental conditions. Thus, the ability of yellow sweet clover to germinate and emerge over a wide range of temperature, pH and salinity, and from depths as great as 8 cm, partially explains why this plant is one of the most important weeds of canola fields in Iran.

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