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Factors Affecting Germination, Emergence and Establishment of *Melilotus indica* (L.) All.

Rupa Dhawan
Department of Agronomy
CCS Haryana Agricultural University, Hisar-125 004 (Haryana), India

ABSTRACT

Laboratory experiments were conducted to study germination, emergence and establishment of yellow sweet clover (*Melilotus indica)*. The seeds showed poor germination in Petri dishes due to a hard seed coat. Mechanical scarification, acid scarification and scarification by a short term exposure (4-5 min) to boiling water resulted in 100% germination of 1-, 2- and 3-year old seeds. Seedling emergence from non-scarified seeds was observed within 15 days. The emergence from surface sown seeds in pots was >80% and the emergence declined significantly with an increase in the seeding depth. Germination percentage, hypocotyl and radical growth of seedlings from seeds scarified by boiling were not inhibited by an osmotic potential upto -0.8 MPa .The growth was, however, inhibited by a salt stress of 40 mM NaCl indicating that the species is drought tolerant and salt sensitive. Plant growth was slow during the initial 60 days i. e. from November end to January end. The information could be exploited for developing strategies for the management of this weed.

Key words : Hot water treatment, moisture stress, salt sensitivity, scarification

INTRODUCTION

Melilotus indica (L.) All. (=*Melilotus indicus* = *Melilotus parviflora* Desf.=*Trifolium indica* (L.)= *Melilotus officinalis* (L.) Pall) is an herbaceous plant belonging to the family Leguminosae (Fabaceae). It is variously called as sweet clover, sour clover, Indian sweet clover, annual yellow sweet clover, small flowered sweet clover, small flowered meliot and small meliot. The herb is a native of northern Africa, Europe and Asia. It is naturalized in rest of the world like Australia and New Zealand and is referred to as King Island meliot. Its common name in northern India is '*Senji'* and '*Jangli Methi*'. It has been reported to cause infestation and competition in winter crops like wheat, mustard, pea and chickpea (Tewari *et al.,* 1997; Nepalia and Jain, 1998; Singh *et al.,* 2003; Chaudhary *et al.,* 2005). It is a major annual weed of winter crops (Singh *et al.,* 1995) in Haryana, while in some other parts of the world it is reported to be a biennial over-wintering the first season in the form of crown buds and in the second season it grows further and reproduces. In Haryana, the plants emerge in the months of November-December when the day/night temperature is 18/5^{O}C and set seeds in March-April at a day/night temperature of 35/18^{O}C (Singh *et al.,* 1995). The plants are erect and 50-100 cm tall. The stem is green, branched, solid and smooth. Leaves are petiolate, alternate and compound trifoliate. Flowers are axillary, small (2-3 mm), yellow in colour and occur in racemes. Fruit is a globose pod, each pod normally contains one seed. At times it may contain 2-3 seeds. Seeds are 1.5 mm, ovate, short, yellow greenish or reddish. These are known to possess hard seed coat and are suspected to persist longer in the soil. *Melilotus indica* resembles white sweet clover (*Melilotus alba*) in growth pattern except for flower colour.

Although some information on germination response of *Melilotus* sp. from European countries is available (Hamly, 1932) but it lacks on *Melilotus indica* in the Indian context. This investigation was, therefore, planned to study (i) the effect of varying environmental factors on seed germination and (ii) the growth and reproductive potential of the weed.

MATERIALS AND METHODS

Seeds of *M. indica* were collected from farms of Haryana Agricultural University, Regional Research Station, Uchani, Karnal in the month of April. Seeds from many plants were pooled, kept in glass bottles in the laboratory and tested for germination. Seeds were collected for three consecutive years i. e. **rabi** seasons of 2003-04 through 2005-06 to compare the viability in the newly shed seeds and old seeds. The germination

tests were carried out at two temperatures i. e. 5±2°C and 18±2°C in an incubator.

Germination Response of Non-scarified Seeds

Twenty seeds from the lot collected in April 2003-04 were placed in three replicates in Petri dishes (6" dia) lined with filter paper and soaked in distilled water in an incubator at 18±2°C in May 2004 and tested for germination at weekly intervals upto four weeks. The test was repeated at monthly intervals for 12 months with three replicates. Seeds with 1 mm radical were considered as germinated.

Effect of Temperature on Imbibition and Germination of Seeds

Twenty (six-month old) seeds were placed in three replicates in Petri dishes with 20 ml distilled water at the two temperatures i. e. 5±2°C and 18±2°C. The number of seeds that imbibed water and showed radical emergence was counted.

Effect of Low Temperature Pre-treatment

The seeds were kept at 5±2°C for 24, 48 and 72 h and later shifted to a temperature of 18±2°C. Germination was recorded after one week.

Effect of Scarification Treatments on Germination

Six-month old seeds (twenty per treatment, in three replicates) were mechanically scarified by breaking the seed coat by rubbing with a sand paper and tested for germination in Petri dishes lined with filter paper soaked in distilled water. Number of seeds germinated was recorded after one week. Seeds were also soaked in dilute sulphuric acid (10 and 50%), concentrated sulphuric acid, 1% solution each of sodium bicarbonate and potassium hydroxide for varying periods (15 min to 1 h). These were washed with water thrice and allowed to germinate in Petri dishes at 18±2°C. Scarification by keeping seed in boiling water for short interval (3-5 min) was also attempted.

Effect of Age of Seeds

Twenty seeds in three replicates from 1-, 2- and 3-year old lots were tested for germination response after mechanical scarification, acid scarification and scarification by boiling in Petri dishes lined with filter paper and kept at 18±2°C.

Effect of Salt Stress and Osmotic Stress on Germination of the Weed

Six-month old seeds scarified by boiling method were used for studying germination response under moisture and salt stress in 2006. To determine the effect of salt stress, seeds were kept in Petri dishes containing 0, 40, 80, 120 and 160 mM NaCl solutions. To evaluate the effect of osmotic stress, solutions with osmotic potentials of 0, -0.2, -0.4, -0.6, -0.8, - 1.0 MPa were prepared by dissolving polyethylene glycol 6000 in distilled water (Burlyn and Kaufmann, 1973). Number of seeds germinated was recorded after seven days.

Effect of Seed Burial Depth on Emergence of the Weed

The effect of seed burial depth on seedling emergence was studied in pots in a screen house in 2007. One hundred non-scarified seeds were placed in sandy loam soil in earthen pots (6" dia). Seeds were placed on the soil surface and 2.0, 4.0, 6.0 and 8.0 cm depth in pots in three replicates. Soil in the pots was moistened from the top as and when necessary. Seedlings were considered emerged when cotyledons emerged and the experiment was terminated when no further emergence was recorded for continuous seven days.

All the above experiments were repeated with parallel results. Analysis of variance was calculated on the data obtained in one experiment with three replicates. Regression analysis on the data for osmotic stress and salt stress was also performed.

Growth and Reproductive Potential of the Weed

To study the growth behaviour of the weed, seeds were allowed to germinate in pots (9" dia) filled with sandy loam soil in November end in 2007 and 2008. Two plants per pot were maintained in three replicates to study the growth behaviour. Data on plant height and leaf number/plant were recorded at periodic intervals. Time to flower and fruit initiation was also recorded. Number of flowers/plant and number of fruits/plant were

recorded at the time of harvest. Since the growth behaviour was similar in the two years', data for only 2007 were presented. Standard error of means was calculated and regression trends were plotted.

RESULTS AND DISCUSSION

Germination Response of Non-scarified Seeds

Non-scarified seeds when allowed to germinate at a temperature of 18±2°C showed 10% germination in the initial three months i. e. from May to July. Later, the germination improved to 20% in August to April (Table 1).

Table 1. Germination response of non-scarified seeds tested at monthly intervals at a temperature of 18±2°C for four weeks

Month	Per cent germination				
	1st week	2nd week	3rd week	4th week	Mean
May	0	10	10	10	7
June	0	10	10	10	7
July	0	10	10	10	7
August	0	20	20	20	15
September	0	20	20	20	15
October	0	15	15	15	10
November	0	15	20	20	13
December	0	10	15	15	10
January	0	10	20	20	12
February	0	15	20	20	13
March	0	10	20	20	12
April	0	10	10	20	10
Mean	0	12	15	15	

LSD (P=0.05) : Duration of imbibition : 0.19, Month of test : 0.34, Duration x Month : 0.68

Data are mean of three replicates.

Effect of Temperature on Imbibition and Germination of Seeds

The number of seeds that imbibed water at 5±2 ^{O}C was higher as compared to those that imbibed water at 18±2°C. While 10% seeds imbibed water at 5±2°C after one week, only 2% seeds imbibed water at 18±2^{O}C. The number increased to 70% at a temperature of 5±2°C and to 10% at 18±2°C (Fig. 1). Some of the seeds showed radical emergence which did not grow further.

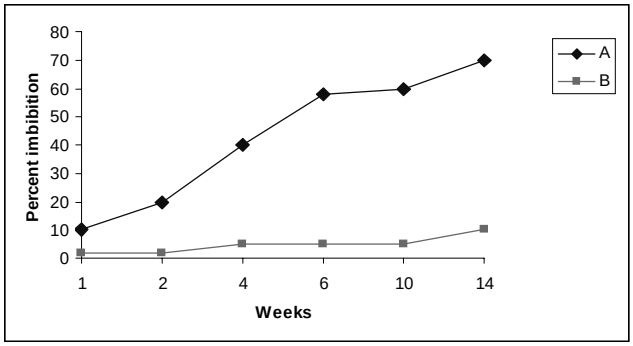

Fig. 1. Effect of temperature (A represents 5±2^{O}C; B represents 18±2^{O}C) on imbibition by *M. indica* seeds upto 14 weeks. LSD (P=0.05) : Number of weeks=3.5, Temperature=2.2 and Weeks x Temperature=5.2.

Effect of Low Temperature Pre-treatment

A pre-treatment of the seeds at 5±2°C for 24 or 48 h and transfer to 18±2°C did not result in germination. However, a low temperature exposure for 72 h and then transferred to 18±2^{O}C resulted in 50-60% germination of the seeds (Table 2).

Table 2. Germination response of the seeds at a temperature of 18±2°C after a pre-treatment at 5±2°C for 24-72 h

Pre-treatment	Duration (h)	Per cent germination at 18±2°C				
		1 week	2 weeks	3 weeks	4 weeks	Mean
5±2°C	24	0	0	0	0	0
5±2°C	48	0	0	0	0	0
5±2°C	72	0	0	50	60	27
Mean		0	0	16	20	

LSD (P=0.05) : Weeks : 1.35, Duration of low temperature : 1.17, Weeks × Duration : 2.3

Data are mean of three replicates.

Germination Response after Scarification

Per cent germination in the range of 95-98 was observed in mechanically scarified seeds (Table 3). Treatment with dilute sulphuric acid (1-50%) for 5 min to 1 h and concentrated sulphuric acid for 5 to 30 min resulted in only 5% germination after two weeks. However, a 45-min treatment with concentrated sulphuric acid resulted in complete scarification and 100% germination of the seeds. The radical that emerged did not grow further and decayed possibly due to the absorption of the acid during imbibition. Alkali treatment (1% sodium bicarbonate or 1% sodium hydroxide) was

Table 3. Germination response of 6-month old seeds as affected by different scarification treatments

Treatment	Duration of treatment (min)	Per cent germination at 18± 2°C				
		Number of weeks of imbibition				
		1	2	3	4	Mean
Control		0	0	0	0	0
Mechanical pressure	1-5	95	95	98	98	96
Sulphuric acid (10%)	45	0	5	5	5	3.7
Sulphuric acid (50%)	45	0	5	5	5	3.7
Sulphuric acid (Conc.)	5	0	0	0	0	0
Sulphuric acid (Conc.)	45	100	100	100	100	100
Sod. bicarbonate (1%)	45	10	10	10	10	10
Pot. hydroxide (1%)	45	0	0	0	0	0
Boiling water (100°C)	3-5	100	100	100	100	100
Mean		33	35	35	35	
LSD (P=0.05) : Scarification : 1.34, Weeks : 0.89, Scarification x Weeks : NS						

Data are mean of three replicates. NS–Not Significant.

not effective as scarification treatment. Leaving the seeds in boiling water for 2-5 min and then shifting to 18±2°C resulted in 100% germination.

Effect of Age of Seeds

1-, 2- and 3-year old seeds showed 95-100% germination within a week irrespective of the mode of scarification (Table 4).

Emergence of the Seedlings

The surface sown seeds showed greater emergence after two weeks as compared to the seeds sown at a depth of 2.0-8.0 cm. After six weeks, 86% seedlings emerged from the seeds sown at the surface as compared to 29% emergence from a depth of 2 cm. The emergence was lesser from a depth of 4.0-8.0 cm even after six weeks (Fig. 2).

Table 4. Effect of scarification treatments on 1-, 2- and 3-year old seeds of *Melilotus indica*

Scarification treatment	Per cent germination at 18±2°C after one week			
	Age of the seeds			
	1 year	2 years	3 years	Mean
Control	0	0	0	0
Mechanical scarification	95	98	98	97
Concentrated sulphuric acid (45 min)	100	100	100	100
Boiling water (2-3 min)	100	100	100	100
Mean	73	74	74	
LSD (P=0.05) : Scarification : 1.17, Age of the seed : NS, Scarification × Age : NS				

Data are mean of three replicates. NS–Not Significant.

The data are indicative of the fact that *M. indica* seeds possessed dormancy due to the presence of hard seed coat. Two and three-year old seeds were 100% viable as seen by scarification treatments. In pot culture studies, seedlings emerged from unscarified seeds within 15 days indicating that the seeds might be scarified by soil abrasion, microbial attack or alternating low/high temperatures. Stimulation of germination by alternating low/high temperatures in this species has been emphasized by Turkington *et al.* (1978). Hartley (1971) and Spira and Wagner (1983) reported viability in some *Melilotus* sp. seeds upto 80 and 183 years old seeds of *Melilotus* sp. Higher emergence of the seedlings from the surface sown seeds is indicative of the fact that the

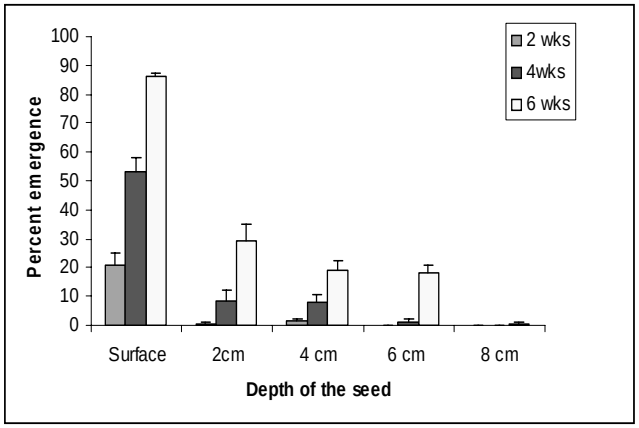

Fig. 2. Emergence of *M. indica* seedlings from different depths (Bars indicate S. E. of the mean of three replicates). LSD (P=0.05) : Number of weeks=2.7, Seeding depth=3.6 and Weeks x Depth=6.2.

tillage practices should be manipulated in such a way that seeds present in deeper layers do not come to surface zone. Stimulation of germination of *Melilotus* species by fires and kill the seedlings emerged by non-selective herbicides before the sowing of the crop is adopted in many countries (Kline, 1984). Scarification by an exposure to boiling temperatures is indicative of the capacity of the seeds to tolerate high temperature. Burning of wheat and rice straw which is a practice in Haryana could also possibly stimulate scarification of *Melilotus* seeds.

Effect of Osmotic Potential and Salt Stress on Seedling Growth

Germination percentage as well as seedling growth of *M. indica* remained unaffected upto an O. P. of -0.8 MPa and declined thereafter (Fig. 3). A decline in germination percentage of many weed species starts at an osmotic potential of -0.6 MPa (Koger *et al*., 2004; Tenton *et al*., 2004; Boyd and Acker, 2004, Dhawan, 2005, 2007; Chauhan and Johnson, 2007, 2008). The data are indicative of the fact that the species is drought tolerant and these ecotypes could be exploited in vegetating the drought prone areas. A salt stress of 100 mM NaCl resulted in a decline in germination percentage of the seeds. Hypocotyl and radical elongation were restricted at a salt stress of 40 mM NaCl or more (Fig. 4) indicating that the species is salt sensitive. The weed species appears to possess a wide adaptability to salt stress. Similarly, the *M. indica* populations from Spain showed sensitivity to salt stress of 50 mM NaCl

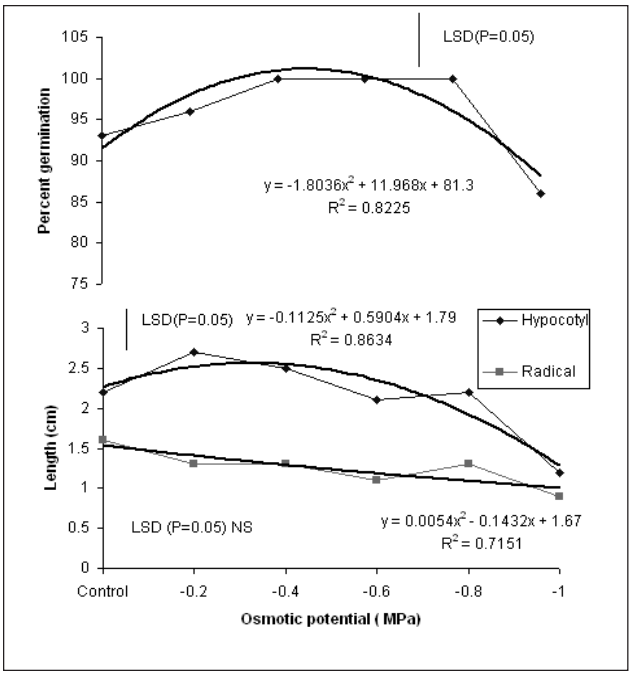

Fig. 3. Effect of osmotic potential on germination of scarified seeds of *M.indica* 15 days after incubation at 18±2°C. Bars indicate standard error of mean of three replicates.

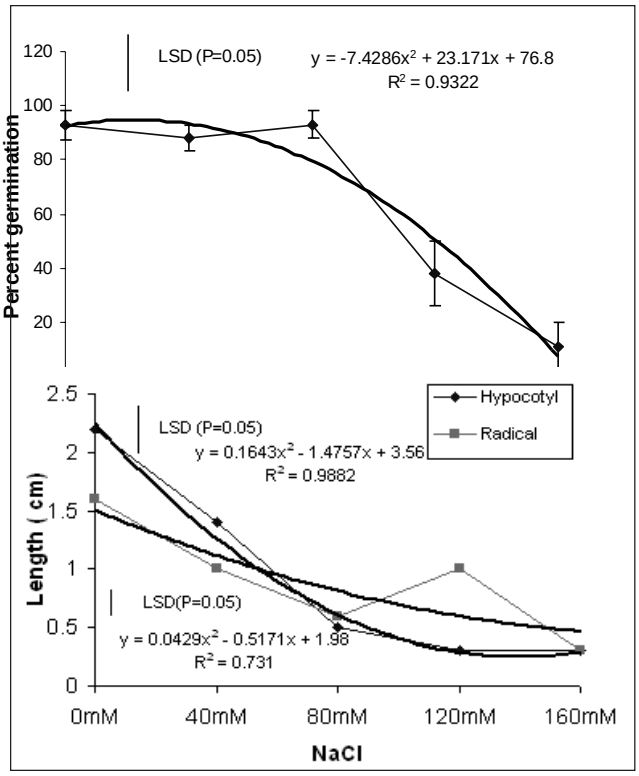

Fig. 4. Effect of NaCl concentration on germination of scarified *M. indica* seeds 15 days after incubation at 18±2°C. Bars represent standard error of mean of three replicates.

(Maranon *et al.*, 1989). While contrary to this populations from Australia showed tolerance upto a salt stress of 200 mm NaCl (Rogers *et al.*, 2008).

Growth and Reproductive Potential of the Weed

The initial growth in terms of plant height and leaf number was slow upto 60 days after sowing (DAS) (Fig. 5). Plants attained a height of 60±6, 150 DAS. The leaf number increased to approximately 135/plant till the time of harvest. Number of primary branches started to increase 45 DAS. Flowering initiated 80-82 DAS and 50% flowering was achieved by 90 DAS. Fruit formation started 15-20 days after flower initiation. The number of fruits per plant was in the range of 1500±166. Seed production per plant was estimated to be in the range of 1125-16,000. One thousand seed weight was 4.75±0.14 g. The initial period of slow growth could be exploited for management of the weed by chemical and cultural means providing competitive edge to the crop.

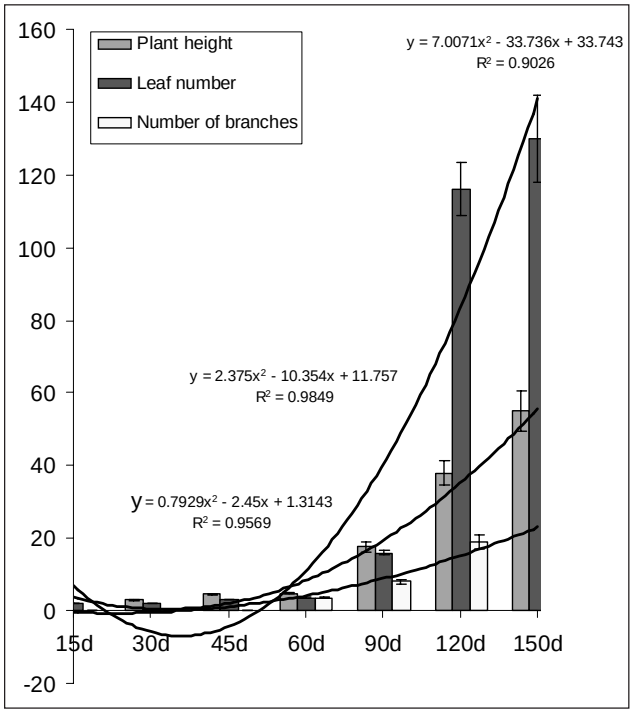

Fig. 5. Periodic changes in plant height (cm), number of leaves and number of branches of *M. indica* (Bars indicate S. E. of mean of five replicates).

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