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FACTORS AFFECTING SEED GERMINATION AND SEEDLING EMERGENCE OF SHEEP SORREL (*RUMEX ACETOSELLA*)

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ABSTRACT

Sheep sorrel (*Rumex acetosella*) is a common weed in farming system in Northern Iran. There is a lack of information on the factors affecting sheep sorrel seed germination. Experiments were conducted under greenhouse and laboratory conditions to investigate the effects of some factors (gibberellic acid (GA_3), cytokinin, scarification, ethanol, pH, osmotic and salt stress and planting depth) on germination and seedling emergence of sheep sorrel. Adding GA_3 in a range of 0 to 200 ppm had pronounced effects on seed germination. In contrast to wet pre-chilling, dry pre-chilling markedly increased germination of sheep sorrel seed compared with control. Sheep sorrel germination was inhibited by high levels of ethanol. Germination decreased from 87% to 39% as salt concentration increased from 0 to 160 mM. Seed germination was sensitive to osmotic stress and was completely inhibited at osmotic potential greater than -0.25 MPa. Germination of sheep sorrel seed was occurred in a wide range of buffered pH solutions but the highest germination occurred over a pH range of 6 to 7. Sheep sorrel seedling emergence was at its maximum on the soil surface, and no seedling emerged from a soil depth of 4 cm. Information gained in this study could be effective in a better understanding of germination requirements and seedling emergence that may be appropriate to an integrative weed control program.

Key words: *Rumex acetosella* germination, burial depth, osmotic stress, salt stress, pH, pre-chilling, scarification.

INTRODUCTION

The genus of *Rumex* (Polygonaceae) contains nearly 20 species that are mostly perennials. The plants of this genus grow widely in wetlands and tolerate a wide range of soil conditions, but they are more common on acidic and low nutrient soils. Sheep sorrel (*Rumex acetosella*) is a problematic weed in wasteland, crops and orchards in northern Iran (Karimi, 2001). Seed germination is a key event in plants life cycle. Several environmental factors such as temperature, light, pH, and soil moisture have been known to affect germination of seeds (Chachalis and Reddy; 2000; Koger et al., 2004). Optimum factors affecting germination are mostly weed species dependent (Burke et al., 2003; Mennan and Ngouajio, 2006). Soil moisture is an important factor that determines seed germination and seedling emergence (Cardwell, 1984).

Balyan and Bhan (1986) reported weed seed germination from different burial depths. Information about the factors affecting germination and seedling emergence provides some insights into the potential ability of weed species to spread into new areas (Kriticos et al., 2003) and also improves management systems for specific weed species control (Chejara et al., 2008). Little information exists on the factors affecting seed germination and seedling emergence of sheep sorrel.

Therefore, the objectives of this study were to determine the effect of gibberellic acid, cytokinin, scarification, pre-chilling, salt and osmotic stress, pH, and burial depth on germination and seedling emergence of *Rumex acetosella*.

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MATERIAL AND METHODS

Seed materials

Seeds of sheep sorrel were collected from fields near Qaemshahr, Mazandaran in September 2010. Seeds were sieved to remove any extraneous materials. The 1000-seed weight was determined (0.40 g) and seeds were stored at room temperature in paper bags until required. Experiments were carried out at the Agricultural Department of Payme-Noor University, Mashhad, Khorasan Razavi, Iran, during August and October 2011.

General germination test

Seeds were tested for viability using 1% tetrazolium chloride solution before starting experiments. For seed germination tests, fifty seeds of sheep sorrel were placed on Whatman No. 1 filter paper in a 9-cm plastic Petri dish. The filter papers were moistened with either 5 ml of distilled water or treatment solutions. All dishes were sealed with Parafilm to reduce loss of water and were placed in a growth chamber (CLC222 Model, Climacell, Germany). The photoperiod was set at 16/8 h (day/night) for all germination tests. Light was provided by fluorescent lamps to produce a light intensity of 300 μmol m^{-2} s^{-1}. The thermo period was set at 25/15°C (day/night) for all germination tests. Seed germination was determined 4 wk after incubation. Seeds were considered germinated when radicles emerged from the seed coat.

Effect of gibberellic acid (GA_3) and cytokinin on germination

To evaluate the effect of GA_3 (FLUKA, Germany) on germination, seeds were placed on filter papers soaked with different GA_3 concentrations of 0, 25, 50, 100, 200 and 400 ppm. The effect of Cytokinin (FLUKA, Germany) on seed germination of sheep sorrel was studied by putting seeds on filter papers soaked with solutions of different concentrations of 0, 0.1, 1 and 5 mM.

Effect of wet and dry pre-chilling duration on germination

Seeds were placed either between two layers of paper towels moistened with distilled water (wet pre-chilling) or dry paper towels (dry pre-chilling) then placed in plastic bags. Samples were stored in a fridge (4-2°C) for 15, 30 and 45 days.

Effect of mechanical scarification duration on germination

Seeds were scarified for 0, 1, 2 and 3 min by rubbing between two layers of sandpaper.

Effect of ethanol concentration on germination

To evaluate the effect of ethanol 95% (MERCK, Germany) on germination, sheep sorrel seeds were put on filter papers soaked with varied ethanol concentrations of 0, 0.3, 3, 15 and 30%.

Effect of salt stress on germination

The effects of salt stress on germination were evaluated by incubating seeds in different sodium chloride (NaCl) (MERCK, Germany) solutions of 0, 10, 20, 40, 80 and 160 mM.

Effect of osmotic stress on germination

To evaluate the effect of osmotic stress on seed germination, aqueous solutions with osmotic potentials of 0, -0.25, -0.5, -1 and -1.5 MPa were prepared by dissolving 0, 99.4, 157.1, 222.2, 314.2 and 384.8 g polyethylene glycol 8000 (MERCK, Germany) in 1 L of distilled water, respectively (Michel, 1983).

Effect of pH on germination

The influence of pH on seed germination was determined by using buffer pH solutions of 4 to 10 prepared according to the method described by Chachalis and Reddy (2000).

Effect of seed burial depth on seedling emergence

The effect of seed burial depth on seedling emergence was studied in a glasshouse by using 15 cm diameter plastic pots. Fifty seeds of sheep sorrel were place on soil surface and then covered with soil to achieve different burial depths of 0, 0.5, 1 and 4 cm. Autoclaved soil (clay 5%, silt 6%, sand 89%; pH 7.8, organic carbon 1.5%) was used for this experiment. Pots were irrigated as

needed to maintain soil moisture at about field capacity. Glasshouse temperature was set up at 26/17 °C (day/night) with a natural photoperiod. Seedling emergence was defined as the appearance of the cotyledons. Emerged seedlings counting were run until 4 weeks after planting.

Statistical analyses

All experiments were conducted in a complete randomised design (CRD) with four replicates. The analysis of variance was performed on the transformed (Arcsin transformation) data obtained as percentage of germination. Significant differences among treatment means were compared by a protected LSD test (P=0.05). Means were separated by standard error bars. Regression analysis was used to determine the response of data for the salt stress experiment.

A functional three parameter logistic model (Kleemann et al., 2007) was plotted to the seed germination rates (%) at different osmotic potential levels. The model fitted was:

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

where: G indicates the total seed germination (%) at osmotic potential x, G_{max} is the maximum seed germination (%), x_{50} is the osmotic potential for 50% inhibition of the maximum seed germination, and G_{rate} shows the curve slope.

RESULTS

Effect of GA_3 and Cytokinin

The effects of two growth regulator on seed germination were investigated in this study. Seed germination increased as GA_3 concentration increased from 0 to 200 ppm, it then decreased when GA_3 concentration increased to 400 ppm (Figure 1). Seed germination of sheep sorrel was markedly stimulated by different cytokinin concentrations. A sharp increase in the final germination percentage with increasing the cytokinin concentrations was recorded. The maximum germination occurred in a range of cytokinin concentrations from 1 to 5 mM (Figure 2).

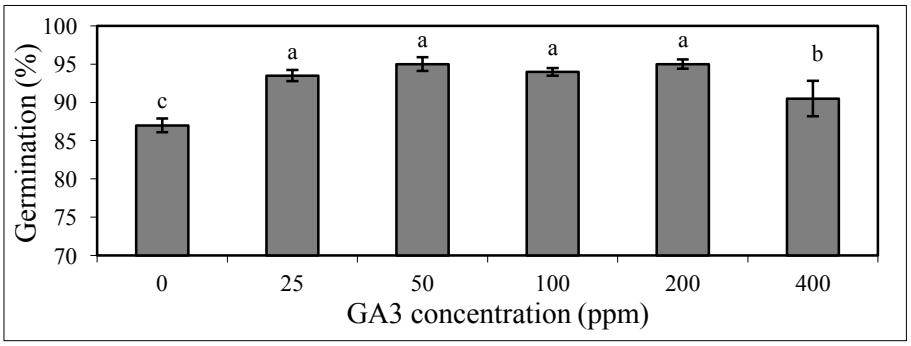

Figure 1. Effect of GA_3 concentrations on sheep sorrel seed germination (Vertical bars represent standard errors of the means.)

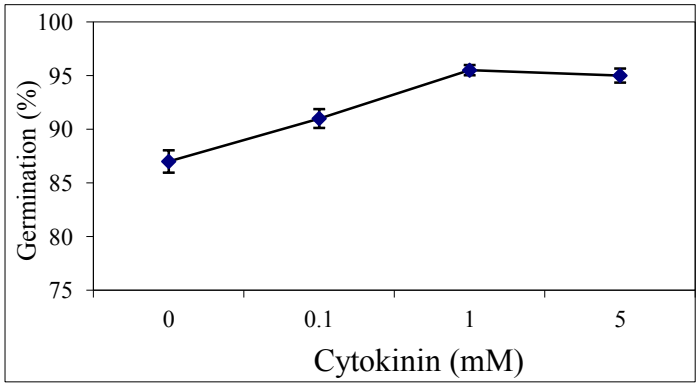

Figure 2. Effect of cytokinin concentrations on sheep sorrel seed germination (Vertical bars represent standard errors of the means.)

Effect of wet and dry pre-chilling

Germination of sheep sorrel seeds was significantly promoted by wet and dry pre-chilling (Figures 3 and 4).

Increasing the duration of wet and dry pre-chilling enhanced germination overt the control treatments and maximum seed germination stimulation occurred at 30 days treatment (Figures 3 and 4). However, dry pre-chilling was more effective on promoting seed germination compared to wet pre-chilling (Figures 3 and 4).

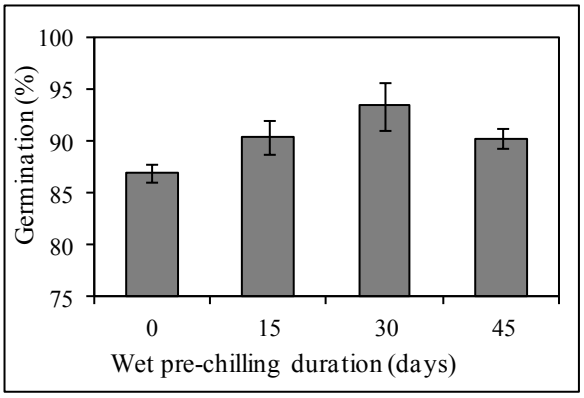

Figure 3. Effect of wet pre-chilling duration on sheep sorrel seed germination
(Vertical bars represent standard errors of the means.)

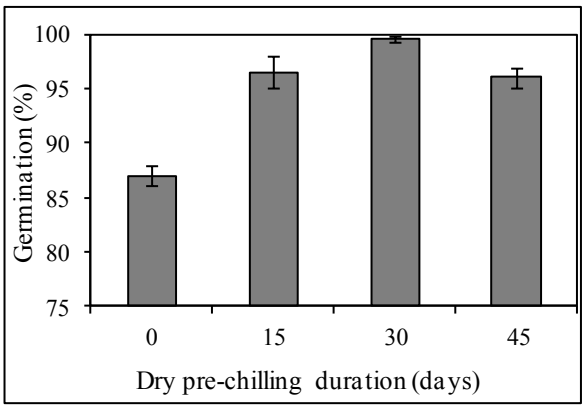

Figure 4. Effect of dry pre-chilling duration on sheep sorrel seed germination
(Vertical bars represent standard errors of the means.)

Effect of scarification duration

Germination of sheep sorrel was affected by scarification. Seed coat scarification for 3 min increased the germination of sheep sorrel dramatically (Figure 5).

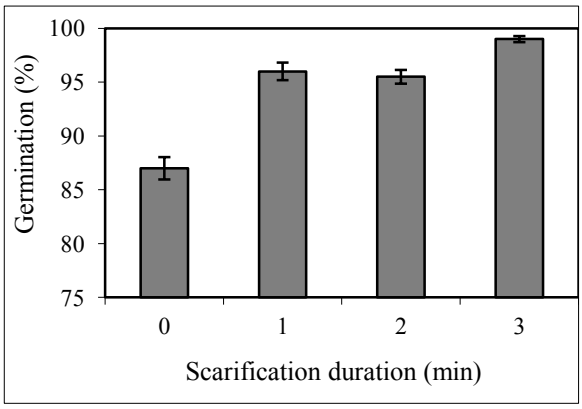

Figure 5. Effect of scarification duration on sheep sorrel seed germination
(Vertical bars represent standard errors of the means.)

Effect of ethanol

Seed germination decreased with increasing in ethanol concentrations. Germination of sheep sorrel was inhibited when ethanol concentrations increased from 3 to 30% (Figure 6). Only 1.5% of seed germinated when ethanol concentration was 3% and no seed germination occurred at ethanol concentrations from 15 to 30% (Figure 6).

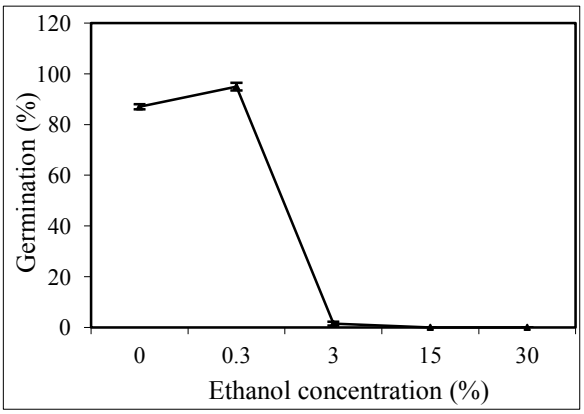

Figure 6. Effect of ethanol concentrations on sheep sorrel seed germination
(Vertical bars represent standard errors of the means.)

Effect of salt stress

A significant reduction in the germination of sheep sorrel seed by increasing in NaCl concentrations was recorded (Figure 7). There was 39% seed germination in the treatment of 160 mM (Figure 7).

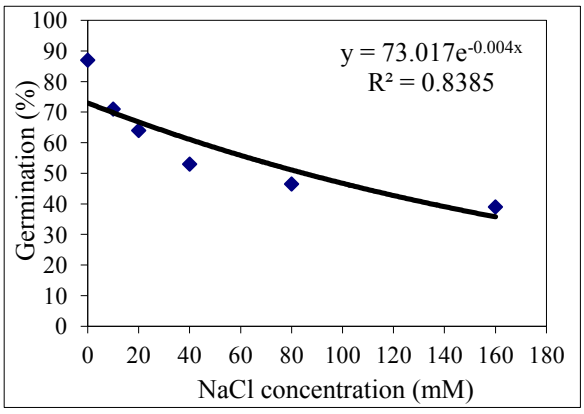

Figure 7. Effect of NaCl concentrations on sheep sorrel seed germination

Effect of osmotic stress

A three-parameter logistic model [$G(\%)=88.25/[1 + (x/0.22)^{25.75}]$, $R^2=0.99$] was fitted to the germination (%) values calculated at different osmotic potential (Figure 8). The osmotic potential required for 50% inhibition of the maximum germination (x50), was -0.22 MPa (Figure 8). Sheep sorrel seeds germination was inhibited at osmotic stress higher than -0.25 MPa (Figure 8).

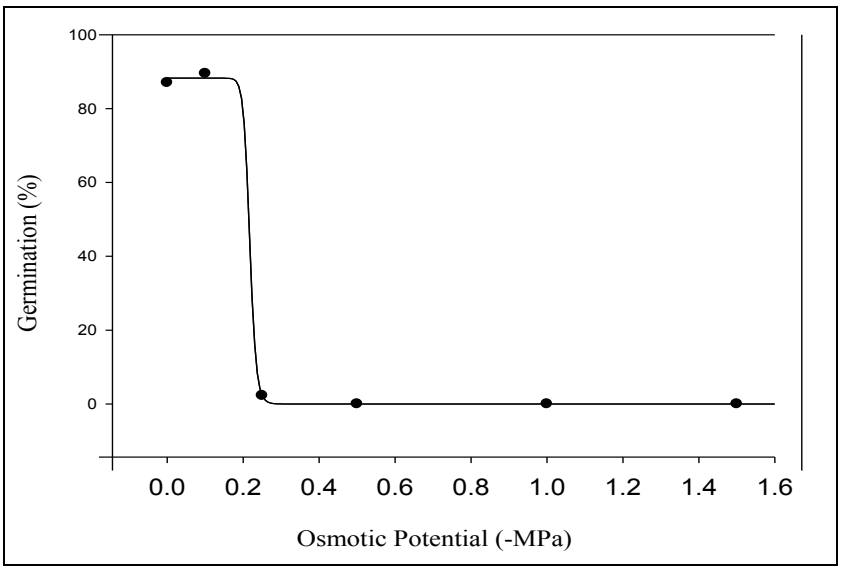

Figure 8. Effect of osmotic potential on sheep sorrel seed germination
[The line represents a functional three-parameter logistic model, $[G (\%) = 88.25/ [1 + (x/0.22)^{25.75}]$, $R^2 = 0.99$.]

Effect of pH

Seed germination of sheep sorrel was affected by buffered pH solutions. The sheep sorrel seed germination was occurred in a wide range of pH (Figure 9). Maximum germination was observed in pH=6-7.

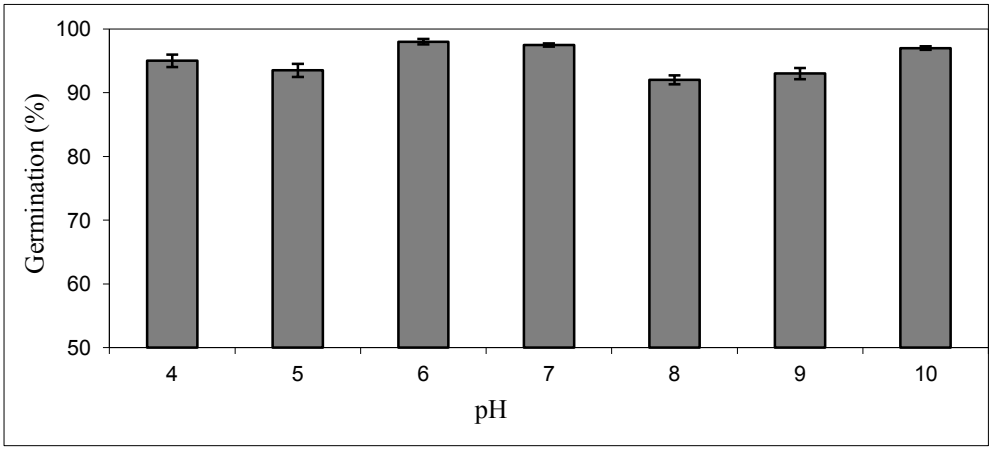

Figure 9. Effect of buffered pH solutions on sheep sorrel seed germination
(Vertical bars represent standard errors of the means.)

Effect of burial depth

The seedling emergence of sheep sorrel was significantly influenced by burial depth (Figure 10). Seedling emergence was maximal (70%) for seeds placed on the soil surface and decreased substantially with the depth of seed burial. No seedlings emergence was observed from seeds buried at a depth of 4 cm (Figure 10).

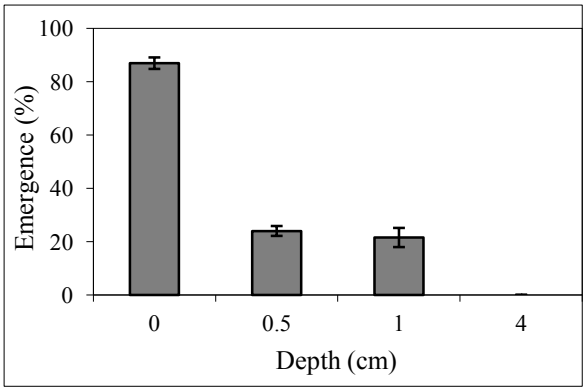

Figure 10. Effect burial depth on sheep sorrel seedling emergence
(Vertical bars represent standard errors of the means.)

DISCUSSION

The results from this study showed that gibberellic acid (GA_3) and cytokinin played crucial roles in sheep sorrel germination. Previous studies indicated that seeds of weed species soaked in GA_3 germinated markedly better than untreated control (Karssen et al., 1987; Malik and Vanden Born, 1987). Karimmojeni et al. (2011) indicated that increasing GA_3 concentration up to 20 ppm enhanced seed germination (29%) of *Lepidium latifolium*, but increasing the concentration of GA_3 more than 20 ppm was not effective in promoting seed germination. For leafy spurge, Foley and Chao (2008) showed that approximately 5 mM of cytokinin induced seed germination. In contrast, Thomas (1992) indicated that cytokinins were not considered for inducing seed germination. Our results are in agreement with the work of Cohn and Butera (1982), Floey and Chao (2005), Balaguera-Lopez et al. (2009) and Wei et al. (2010).

Our results indicated that a period of 30 days pre-chilling would have a pronounced effect on increasing the seed germination of sheep sorrel. The effect of pre-chilling on breaking coat imposing dormancy was reported by Webb and Wareing (1972) in some weed species. Macchia et al. (2001) noted that pre-chilling of *Echinacea angustifolia* seed for 7 or 11 days had no significant effects on germination. However, by increasing in pre-chilling duration to 15 days seed germination increased. Our results are in accordance with the result of Chauhan et al. (2006 d).

Mechanical scarification significantly increased the germination of seeds of sheep sorrel in this study. These results are in agreement with previous studies, which revealed that seed mechanical scarification was an effective method for stimulation of seed germination in different weed species (Chauhan et al., 2006d; Pinto et al., 2007; Wei et al., 2010). Increased germination of scarified seed could be due to increasing imbibition of seed, the movement of inhibitors from within the embryo, or the release from the physical restriction of the seed coat (Makowski and Morrison, 1989).

The results of this study showed that ethanol substantially reduced seed sorrel germination. Nadjafi et al. (2006) reported inhibitory effects of ethanol on the seed germination of *Ferula gummosa* and *Teucrium polium*. Also inhibitory effects of ethanol on seed germination of rice (*Oryza sativa* L.) were reported by Miyoshi and Sato (1997). However, Bewley and Black (1982) reported that ethanol had stimulatory effects on seed germination of some plant species.

Our results indicated that sheep sorrel seeds were able to germinate over a broad range of pH, which indicated that pH was not a limiting factor for germination. Yilmaz and Askoy (2007) reported that the germination of *Rumex scutatus* occurred in a wide range of pH and the highest germination was observed in pH=6-7. Similar results were reported by Chauhan et al. (2006c) in *Sisymbrium orientale* and Chachalis and Reddy (2000) in *Campsis radicans.*

Decreased sheep sorrel seed germination by increasing in NaCl concentrations was also observed in this study. However, our results

indicated that at higher salt stress, some seed of sheep sorrel might germinate. Negative impacts of salt stress on seeds germination of different weeds species were reported by Chachalis and Reddy (2000), Chauhan et al. (2006 c, 2008).

The present results also indicated that sheep sorrel seeds were not able to tolerate short-termed osmotic stress lower than -0.25 MPa. Such a soil moisture condition would occur in mid spring at the mid-life cycle of sheep sorrel in Northern Iran. Different responses to different osmotic potential have been reported in weed species by Ray et al. (2005), Chauhan et al. (2006 b), Kleemann et al. (2007) and Yang et al. (2008).

Decreasing in seedling emergence due to increased burial depth was observed in this study. Similar results have been reported in several weed species by Chachalis and Reddy (2000), Benvenuti et al. (2001 a), Chauhan et al. (2006a, b) and Singh (2012). Benvenuti et al. (2001 b) also indicated that seed germination of *Rumex obtusifolius* decreased by increasing burial depth. Mennan and Ngouajio (2006) noted that lower seedling emergence of seeds buried at deeper depths may be related to their limited seed energy reserves. Higher seedling emergence of surface placed seeds in sheep sorrel may suggest the requirement of light for germination in this weed and indicates that farming practices that provide shallow burial of seeds might induce higher emergence of sheep sorrel seedlings (Chauhan et al., 2006 e).

CONCLUSIONS

Sheep sorrel seed germination was stimulated by increasing in GA_3 and cytokinin concentrations. Germination was tolerant to salt stress, but it was sensitive to drought stress. Scarification duration and dry pre-chilling influenced germination. Ethanol had an inhibitory effect on seed germination. Seeds of sheep sorrel were able to germinate over a broad range of pH between 4 and 10. Seedling emergence was maximal when seed was scattered on the soil surface. A conclusion drawn from the results of this study suggests that farming practices that achieved deep burial of seeds could effectively reduce sheep sorrel population in a field, because of the inability of seep sorrel seedlings to emerge from increased burial depth.

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