

GERMINATION ECOLOGY OF WILD ONION: A RAINFED CROP WEED¹

Ecologia da Germinação de Cebola Selvagem: uma Planta Daninha de Sequeiro

TANVEER, A.², SIBTAIN, M.², JAVAID, M.M.³, and ALI, H.H.⁴

ABSTRACT - *Asphodelus tenuifolius* is becoming a more common weed in rain-fed area in Pakistan. Laboratory and greenhouse experiments were conducted to determine the effect of different environmental factors on germination and emergence of *A. tenuifolius*. Results showed that *A. tenuifolius* can tolerate a wide range of varying environmental factors. Greatest percentage of germination (80%) was recorded at 15 °C constant temperature; however, considerable germination occurred at 20 and 25 °C. Light for 10 h photoperiod stimulate germination of *Asphodelus tenuifolius* compared with complete darkness. Germination was totally inhibited at osmotic stress higher than -0.8 MPa. There was no significant difference in germination at pH 6 to 8; however, there was a slightly decrease at pH 9, compared with distilled water. *Asphodelus tenuifolius* was very sensitive to salinity; however, a few seeds of *A. tenuifolius* were able to germinate even at 150 mM NaCl concentration. Greatest emergence occurred with seed placed at soil surface and emergence decreased with increase in burial depth. No emergence occurred from 4 cm or greater. This information may aid in developing tools and strategies for management.

Keywords: *Asphodelus tenuifolius*, temperature, water stress, salt stress, pH, seedling emergence, seeding depth.

RESUMO - *Asphodelus tenuifolius* está se tornando uma planta daninha cada vez mais comum em plantio de sequeiro no Paquistão. Foram realizados experimentos no laboratório e na estufa para avaliar a influência de diferentes fatores ambientais na germinação e emergência da **A. tenuifolius**. Os resultados mostraram que a **A. tenuifolius** é capaz de tolerar uma ampla gama de fatores ambientais diferentes. A porcentagem máxima de germinação (80%) foi registrada a 15 °C de temperatura constante. No entanto, houve germinação considerável em 20 °C e 25 °C. A inserção de luz em 10 h de fotoperíodo estimula a germinação da *Asphodelus tenuifolius*, o que não ocorre com escuridão completa. A germinação foi totalmente inibida quando houve estresse osmótico maior que -0,8 MPa. Não houve diferença significativa na germinação com pH de 6 a 8, porém, houve uma pequena redução com pH 9, tendo como referência a água destilada. **Asphodelus tenuifolius** se mostrou muito sensível à salinidade, mas, ainda assim, algumas sementes germinaram com concentração de 150 mM de NaCl. Ocorreu maior emergência quando as sementes foram plantadas na superfície do solo. E, na medida em que o enterramento se tornava mais profundo, sua emergência reduzia. Não foram observadas emergências a partir de 4 centímetros de profundidade. Estas informações podem auxiliar no desenvolvimento de ferramentas e estratégias para seu manejo.

Palavras-chave: *Asphodelus tenuifolius*, temperatura, estresse hídrico, estresse salino, pH, emergência de plântulas, profundidade de semeadura.

INTRODUCTION

Wild onion (*Asphodelus tenuifolius*) is a very harmful and noxious weed to chickpea, wheat, mustard, linseed and lentil in Pakistan and

India (Ponia et al., 2001; Tiwari et al., 2001; Nasir & Sultan, 2004), due to its high competitive ability, greater production of seeds and reproduction potential. It is native to the Mediterranean region, but it is widespread,

¹ Recebido para publicação em 23.7.2013 e aprovado em 23.1.2014.

² University of Agriculture Faisalabad, Pakistan, ³ Department of Agronomy, University College of Agriculture, University of Sargodha, Pakistan, <mmjavaid@uos.edu.pk>; ⁴ College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan.



extending from the Mediterranean region east through the Arabian Peninsula to almost 17 countries around the world (Holm et al., 1977). It is an annual herb, 15-50 cm tall with linear, acute, sheathing at the base, with fine puberulous leaves. The plant is eaten raw as well as in cooked form, particularly during famine. The seeds are sharply trigons, and black in colour.

Seed germination, an important factor in determining weed success, is regulated by many environmental factors, e.g. light, temperature, soil pH, soil salinity, weed seed size and available moisture (Koger et al., 2004; Harrison et al., 2007; Chauhan & Johnson, 2008a). Factors like acidity, water and salinity are not limiting; temperature is the major key for successful weed seed germination (Martinkova et al., 2006), and within same species it may be different for varied genotypes (Chauhan & Johnson, 2008a; Harrington, 2009; Wang et al., 2009; Malik et al., 2010; Masin et al., 2010; Gorai et al., 2011). Seeds of some weeds germinate well in the dark and with light; and broad leaf weeds having small seeds are photoblastic (Widderick et al., 2004; Zhou et al., 2005).

Water stress is always a very important attribute to inhibit seed germination (Norsworthy & Oliveira, 2006). Various species behave differently during water uptake in seed imbibition on their germination process (Chauhan & Johnson, 2008b). Ability to germinate under condition of moisture stress may enable weed to take advantage of the condition that limits the growth of other species. The impact of soil moisture on germination varies among weed species; and field condition may differ spatially and temporally, depending on rainfall, temperature, and type of soil. Soil pH is another important environmental factor affecting seed germination of various weeds. Seeds of some weeds can germinate over a broad pH range (Chachalis et al., 2008; Chejara et al., 2008), while other weeds can germinate well in acidic soil conditions (Fried et al., 2008).

Salt stress is a major constraint in crop or weed production worldwide. Physiological processes in some weeds are negatively affected by soil salinity (DiTommaso, 2004; Chauhan & Johnson, 2008b). High salt

concentration inhibits the germination of many weed seeds and it ultimately leads towards death (Koger et al., 2004; Pahlevani et al., 2008; Nemati et al., 2011), with some exceptions as a few types of weed seeds show tolerance to high salt concentrations (Chauhan & Johnson, 2008c). Seed burial depth also affects its germination and seedling emergence (Koger et al., 2004; Mennan & Ngouajio, 2006). Weed seed location in the soil seed bank influences germination, emergence and vertical seed distribution in the soil (Norsworthy & Oliveira, 2006).

Biological and ecological information, specifically germination ecology of a specific weed, is necessary to optimize weed control and maximize the efficiency of management tactics (Bhowmik, 1997). In order to understand the expansion of the geographic range of *A. tenuifolius* in Pakistan, we need to learn how their seeds respond to varied climatic factors. To date, no specific research on the germination ecology of these weeds has been conducted. The objectives of this study are to determine the effects of temperature, light, salt and osmotic stress, pH and burial depth on *A. tenuifolius* seed germination and seedling emergence.

MATERIALS AND METHODS

Seed collection

Experiments were conducted at the Department of Agronomy, University of Agriculture Faisalabad, Pakistan under laboratory and greenhouse conditions during 2009. In March-April 2009 mature wild onion (*A. tenuifolius*) plants were collected from several farmer fields in district Bhakkar (31° N, latitude and 71° E, longitude), Pakistan and a composite sample was prepared. A working sample was drawn from this composite sample. Then seeds were removed and cleaned manually and dried at 25 °C (room temperature) for 20 days and stored in small cloth bags until used in all experiments.

Experimental methodology

Germination was determined by placing 25 seed evenly in a 9 cm diameter Petri plate containing filter paper Whatman No. 10,



moistened with 5 mL distilled water or a treatment solution. Seed of *A. tenuifolius* were surface sterilized by soaking in 10% sodium hypochlorite (NaOCl) for 5 min, followed by five rinses with distilled water before the start of each germination trial. Petri plates were sealed with Para film to reduce the water loss. All the experiments (except for the temperature experiment) were conducted at 15 °C temperature with 10 h photoperiod (except for the light experiment). Fluorescent lamps were used to produce a photosynthetic photon flux density of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for all the experiments, except for the dark treatment in the light experiment. Germinated seed with a radicle at least 2 mm long were counted and removed daily for a period of 3 wk. Petri plates assigned to complete darkness treatment were opened in a dark room with a safe green light.

Effect of temperature

Seeds were incubated at 15, 20 and 25 °C for 3 wk to determine the optimum temperature for germination. Petri plates were kept in a germinator (Seedburo Equipment Company, Chicago, IL, USA) and the temperatures were maintained constant.

Effect of light

To evaluate the effect of light on *A. tenuifolius* germination, seeds were placed in Petri plates and immediately wrapped with aluminum foil to ensure no light penetration. Other treatment was left uncovered to allow 10 h light exposure. Light was provided by fluorescent over-head bulbs set for 10-h light, 14-h dark regime with a photosynthetic photon flux density of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Effect of drought stress

Seed germination of *A. tenuifolius* was checked against drought stress using aqueous solutions of different osmotic potential 0 (Control) -0.2, -0.4, -0.6, -0.8, -1.0 MPa. These aqueous solutions were prepared with PEG 6000 (Polyethylene glycol) by using the Michel & Kaufman (1973) equation as under:

$$\text{Water potential} = - (1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) 18CT + (8.39 \times 10^{-7}) C^2T$$

where C is the concentration of PEG (g kg⁻¹ distilled water) and T is temperature (°C).

After preparing the solutions osmotic potential was confirmed by osmometer (vapour pressure type), model 5520; Wescor, Logan, UT, USA. Control treatment was carried out with distilled water.

Effect of pH

To examine the effect of pH on germination, buffer solutions of pH 6 to 9 were prepared according to the method described by Chacahlis & Reddy (2002). A 2-mM solution of MES [2-(N-morpholino) ethanesulfonic acid] was adjusted to pH 6 with 1 N hydrogen chloride (HCl) or sodium hydroxide (NaOH). A 2-mM solution of HEPES [N-(2-hydroxy-methyl) piperazine-N-(2-ethanesulfonic acid)] was adjusted to pH 7 or 8 with 1 N NaOH. A pH 9 buffer was prepared with 2-mM TRICINE [NTris (hydroxymethyl) methylglycine] and adjusted with 1 N NaOH. Unbuffered deionized water (pH 6.2) was used as a control.

Effect of salt stress

Germination of *A. tenuifolius* seeds was checked against different levels of sodium chloride 50, 100, 150, 200 mM. While in control treatment distilled water was used.

Effect of seeding depth

This experiment was carried out in green house. Pots having 25 cm diameter were filled with sand (0.5 kg per pot). Twenty five seeds of *A. tenuifolius* per pot were placed at soil surface or within the soil from 0 cm (surface placement) 1, 2, 3, 4, 5, 6 cm burial depths. Every pot was initially irrigated with 100 ml of distilled water. Greenhouse temperatures were 15±2 °C during the day and 10±2 °C at night with a 10-h photoperiod (200 to 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Pots were left opened and watered as needed to maintained adequate soil moisture. Each pot has a hole at the bottom for drainage. Seedlings were considered emerged when a cotyledon was visible at the soil surface. Seedling emergence was recorded daily for 21 days.



Germination/emergence index (GI/EI) was calculated by following formula used by Association of Official Seed Analysis (AOSA, 1990).

$$GI \text{ or } EI = \frac{\text{No of germinate or emerged seeds}}{\text{Days of first count}} + \dots + \frac{\text{No of germinated or emerged seeds}}{\text{Days of final count}}$$

It was noted when the germination/emergence of a treatment started, i.e. when first seed of *A. tenuifolius* was germinated or emerged. Coolbear et al. (1984) formula was used to determine time to 50% germination/emergence.

$$T_{50} \text{ or } E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)}$$

where N is the final number of germinated or emerged seed and n_j and n_i are the cumulative number of seed germinated by adjacent counts at times t_j (day) and t_i (day) respectively, when $n_i < N/2 < n_j$.

Mean germination or emergence time (MGT or MET) was calculated according to the equation of Ellis & Roberts (1981):

$$MGT \text{ or } MET = \frac{\sum Dn}{\sum n}$$

where n is the number of seed that had germinated on day D and D is the number of days counted from the beginning of germination experiment. The germination or emergence index (GI or EI) was calculated as described by the Association of Official Seed Analysis (AOSA, 1983) using the following formula:

Statistical analysis

A completely randomized design with four replications was used in all experiments. Data were subjected to analysis of variance (ANOVA) with the use of SAS (2002). The model structure of ANOVA was $Y_{ij} = \mu + T_{i \in ij}$ where Y_{ij} is the observed response variable, μ is an overall mean, T_i is the explanatory variable and

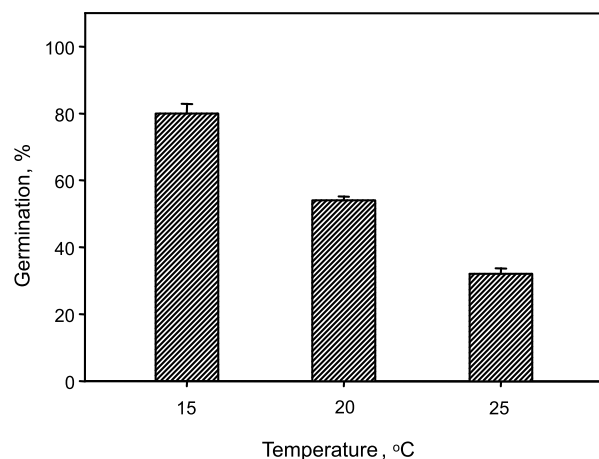
ϵ_{ij} is the error. The significant difference among treatment means were identified by using Fisher, LSD at $P < 0.05$ (Steel et al., 1997). A square-root arcsine transformation was used to stabilize the variances for percentage data before analysis (Bartlett, 1947). Nonlinear regression analysis was used to determine how NaCl, osmotic stress or burial depth affected percentage germination or emergence. Germination (%) values at different concentrations of NaCl and osmotic potential were fitted to a functional three-parameter logistic model using Sigma Plot 2008 (version 11.0). The model fitted was $G (\%) = G_{max} / [1 + (x/x_{50})^g]$, where G is the total germination (%) at concentration x , G_{max} represents maximum germination (%), x_{50} is the NaCl concentration or osmotic potential for 50% inhibition of the maximum germination and g indicates the slope. A three parameter logistic model $\{E (\%) = E_{max} / [1 + (x/x_{50})^e]\}$ was fitted to *A. tenuifolius* seedling emergence (%) obtained at different burial depth of 0 to 6 cm. where E is the total seedling emergence (%) at burial depth x , E_{max} is the greatest seedling emergence (%), x_{50} is the burial depth for 50% inhibition of the greatest seedling emergence and e indicates the slope. The t-test was used to assess significant difference between light and dark treatment in the light experiment.

RESULTS AND DISCUSSION

Effect of temperature

Asphodelus tenuifolius germination was tested against varying temperature regimes from 15 to 25 °C with an interval of 5 °C. *Asphodelus. tenuifolius* germination was significantly affected by different temperatures; and greatest *A. tenuifolius* germination (80%) was recorded at 15 °C (Figure 1). However germination of *A. tenuifolius* decreased with increasing temperature, and lowest (32%) was noted at 25 °C (Figure 1). The starting time for *A. tenuifolius* germination was lowest at 15 °C (2.75 days). However it increased with increasing temperature, i.e. *A. tenuifolius* took 3.00 and 4.00 days at 20 °C and 25 °C, respectively, to start its germination (Table 1). *Asphodelus tenuifolius* took statistically





Vertical bars represent \pm standard error of the mean.

Figure 1 - Effect of temperature on *A. tenuifolius* seed germination (%).

similar days to its 50% germination at 15 °C (5.06 days) and at 20 °C (4.91 days), however *A. tenuifolius* took greatest (7.75 days) at 25 °C. Mean germination time of *A. tenuifolius* was highest (7.40 days) at 25 °C and it was followed by 5.84 days at 15 °C, however lowest (5.57 days) *A. tenuifolius* mean germination time was noted at 20 °C. Maximum (3.86) *A. tenuifolius* germination index was noted at 15 °C and it decreased with increasing temperature i.e. 2.62 at 20 °C and 1.22 at 25 °C (Table 1).

Our findings show that 25 °C temperature and above have negatively affected *A. tenuifolius* germination; and that chances of heavy *A. tenuifolius* infestation during chickpea crop emergence in October are less likely because temperature is about 25.9 °C in October. However, as temperatures decrease in November, December and January

(20 to 13 °C), *A. tenuifolius* germination in chickpea may increase. Many researchers observed a significant effect of temperature on weed seeds germination (Kabat et al., 2007; Guma et al., 2010). Seed germination of *Leptochloa chinensis* and *Cuscuta campestris* was highest at 25 °C and 30 °C, respectively (Benvenuti et al., 2004, 2005), however *C. campestris* seed germination was completely inhibited at 10 °C. Similarly, Ingolia et al. (2008) observed 60 to 80% seed germination of *Rorippa subumbellata* at 24 °C. Seeds of coolatai grass were reported to germinate over a wide range of temperature range from 5 to 45 °C; however germination percentage was very low above 40 °C (Chejara et al., 2008).

Effect of light

Light in 10 h photoperiod resulted in highest germination percentage (87%) of *A. tenuifolius* compared with complete darkness (0 hours light) that resulted in 78% germination. Table 2 showed a non-significant effect of dark and light on *A. tenuifolius* time to start germination, time to 50% germination and mean germination time. However statistically significant and highest germination index (5.21) of *A. tenuifolius* was observed under light and lowest (4.54) was observed under darkness. The results of our experiments show that darkness did not significantly reduce *A. tenuifolius* germination and 78% germination under darkness shows that *A. tenuifolius* is less sensitive to darkness (0 hours of light) for its germination. It suggests that *A. tenuifolius* seeds can germinate well under dense crop canopy or under shade conditions, and this ability of *A. tenuifolius* seeds can pose a major threat to crop production. In contrast, light sensitivity of

Table 1 - Effect of varying temperature regimes on starting time of germination, time to 50% germination, mean germination time and germination index of *A. tenuifolius*

Temperature	T-start \pm SE (days)	T-50 \pm SE (days)	MGT \pm SE (days)	GI \pm SE
15 °C	2.75 b \pm 0.25	5.06 b \pm 0.12	5.84 b \pm 0.08	3.86 a \pm 0.10
20 °C	3.00 b \pm 0.00	4.91 b \pm 0.06	5.57 c \pm 0.02	2.62 b \pm 0.05
25 °C	4.00 a \pm 0.00	7.75 a \pm 0.14	7.40 a \pm 0.10	1.22 c \pm 0.06
LSD \leq 0.05	0.46	0.36	0.24	0.24

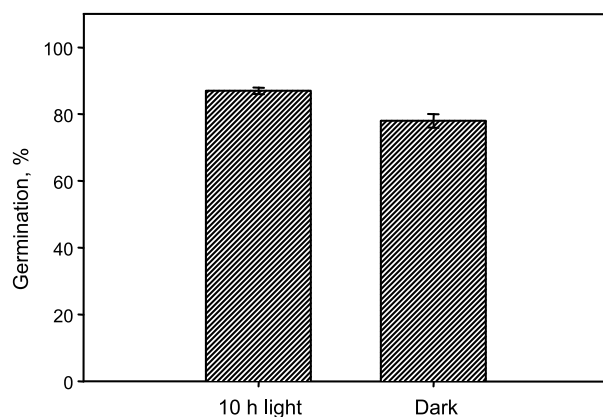
Values with same letters did not differ significantly at $P \leq 0.05$. T-start = starting time of germination, T-50 = time to 50% germination, MGT = mean germination time, GI = germination index and SE = standard error.



Borreria ocymoides (purple leaf button) (Chauhan & Johnson, 2008e), *Amaranthus spinosus* (spiny amaranth) (Chauhan & Johnson, 2009c) and *Synedrella nodiflora* (Chauhan & Johnson, 2009b) was reported in different studies. Similar to our results Rao et al. (2008) and Wang et al. (2009) reported that germination of *Beckmannia syzigachne* (American sloughgrass) and *Urena lobata* (cadillo) seeds was not affected by darkness or light. Seeds of *Eluesine indica* (goosegrass) germinated well (68.72%) under 12 hours of light and dark, compared with complete darkness (17-25%) (Chauhan & Johnson, 2008d).

Effect of drought stress

Asphodelus tenuifolius seed germination decreased from 87 to 8% as drought stress increased from 0 to 0.6 MPa. No germination of *A. tenuifolius* seeds was observed at 0.8 MPa osmotic potential. Logistic model showed 50% inhibition in germination of *A. tenuifolius* seeds at -0.38 MPa osmotic potential (Figure 3).



Vertical bars represent ± standard error of the mean.

Figure 2 - Effect of dark/light on *A. tenuifolius* seed germination (%).

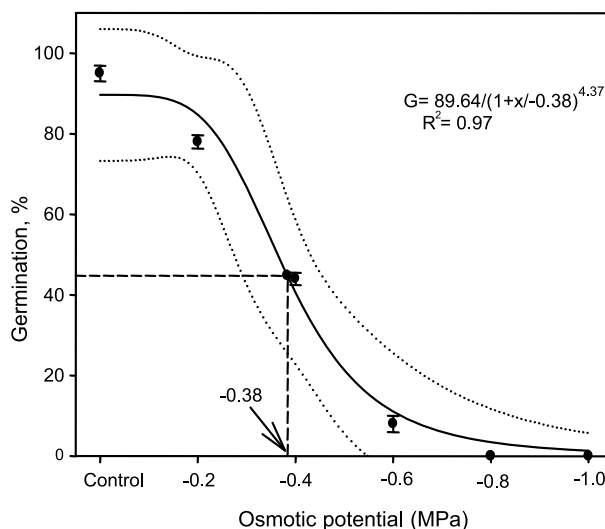
Table 2 - Effect of dark/light on starting time of germination, time to 50% germination, mean germination time and germination index of *A. tenuifolius*

Light	T-start ± SE (days)	T-50 ± SE (days)	MGT ± SE (days)	GI ± SE
10 h light	2.00 ± 0.00	4.71 ± 0.20	6.00 ± 0.15	5.21 a ± 0.07
0 h light	2.00 ± 0.00	4.92 ± 0.25	5.96 ± 0.16	4.54 b ± 0.07
LSD ≤ 0.05	NS	NS	NS	0.24

Values with same letters did not differ significantly at P≤0.05. NS = non-significant, T-start = starting time of germination, T-50 = time to 50% germination, MGT = mean germination time, GI = germination index and SE = standard error.

Increase in time to start *A. tenuifolius* germination was noted with increased osmotic stress (From 0 to -0.6 MPa) and *A. tenuifolius* seeds took most days (6.75 days) to start its germination at 0.6 MPa osmotic stress (Table 3). Similar trend in time to obtain 50% germination and MGT was observed and *A. tenuifolius* took most days (9.94 and 8.81 days) at -0.6 MPa, respectively (Table 3). Table 3 showed that (GI) decreased with increased levels of drought stress. The highest G.I was recorded with control (3.74) and lowest was recorded at -0.6 MPa drought stress (0.27).

Our results showed that moisture availability or rainfall is very important for *A. tenuifolius* germination, and that drought can adversely decreased *A. tenuifolius*



Vertical bars represent ± standard error of the mean. Vertical dash line represents X-axis value at 50% of greatest germination.

Figure 3 - Effect of water availability on *A. tenuifolius* seed germination (%). The bold lines represent a three-parameter logistic model fitted to the data and dotted lines show 95% confidence intervals.



Table 3 - Effect of water availability on starting time of germination, time to 50% germination, mean germination time and germination index of *A. tenuifolius*

Drought stress (MPa)	T-start \pm SE (days)	T-50 \pm SE (days)	MGT \pm SE (days)	GI \pm SE
0 (Control)	5.00 c \pm 0.00	6.16 b \pm 0.05	6.57 b \pm 0.03	3.74 a \pm 0.07
-0.2	5.00 c \pm 0.00	9.31 a \pm 0.18	9.52 a \pm 0.11	2.21 b \pm 0.09
-0.4	6.00 b \pm 0.00	9.29 a \pm 0.34	9.78 a \pm 0.10	1.19 c \pm 0.10
-0.6	6.75 a \pm 0.25	9.94 a \pm 1.36	8.81 a \pm 0.69	0.27 d \pm 0.07
-0.8	N/G	N/G	N/G	N/G
-1.0	NG	N/G	N/G	N/G
LSD \leq 0.05	0.39	2.18	1.08	0.25

Values with same letters did not differ significantly at $P \leq 0.05$. N/G = non-germinated, T-start = starting time of germination, T-50 = time to 50% germination, MGT = mean germination time, GI = germination index and SE = standard error.

germination. We further conclude that during the drier chickpea growing months *A. tenuifolius* germination would be restricted to low laying areas, where more moisture of summer rainfall is preserved in August-September. Similar response for seed germination of *Eupatorium adenophorum* (crofton weed); (Lu et al., 2006), *Synedrella nodiflora* (Chauhan & Johnson, 2008b) and *Eluesine indica* (goosegrass) (Chauhan & Johnson, 2008d) was recorded, i.e the seeds of these weeds are sensitive to different levels of drought stress.

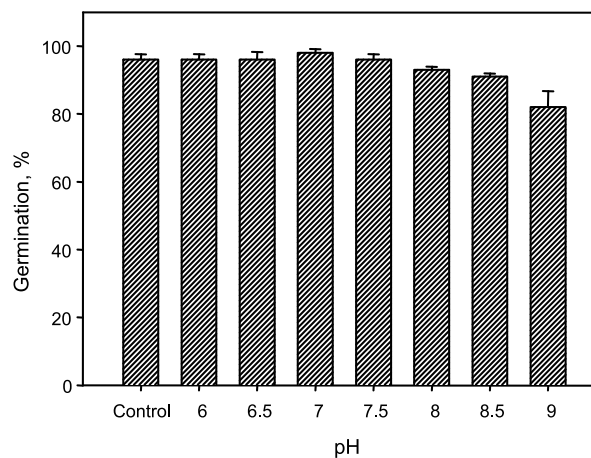
Contrary to the above findings, seed germination of *Hibiscus trionum* (venice mallow); (Chachalis et al., 2008), *Rapistrum rugosum* (turnip weed); (Chauhan et al., 2006c) and *Solanum sarrachoides* (hairy night shade) (Zhou et al., 2005) was recorded under drought stress (low water potential). Similarly, in the studies of Chauhan & Johnson (2008b), seed germination of indian crabgrass was inhibited at higher levels (-1.0 MPa) of water stress, however southern crabgrass seeds germinated to some extent.

Effect of pH

Different levels of pH had a significant effect on *A. tenuifolius* seed germination and other germination related parameters. *Asphodelus tenuifolius* seeds exhibited highest (98%) germination at pH 7. *Asphodelus tenuifolius* seeds showed similar germination of 96% at pH levels of 6.00, 6.20, 6.50 and 7.50. Lowest germination of *A. tenuifolius* (82%) was recorded at pH 9.00 (Figure 4). Starting time

of *A. tenuifolius* germination increased with increasing pH levels from 6.00 to 7.5 (2.00 to 5.00 days), however pH increase from 7.5 to 9.00 did not affect the starting time of *A. tenuifolius* germination (5.00 days). *Asphodelus tenuifolius* seeds took the lowest time to 50% germination (3.91 days) and MGT (5.36 days) with control (pH 6.2); however maximum time was taken at pH levels of 7.5 and 8.00. The highest GI (5.87) was measured with control and the lowest was measured at pH level 9.00 (2.82). However, pH levels of 7.5, 8.00, 8.5 and 9.00 exhibited statistically similar GI of *A. tenuifolius* seeds (Table 4).

Our findings regarding the effect of pH on *A. tenuifolius* germination showed that *A. tenuifolius* can germinate under a wide



Vertical bars represent \pm standard error of the mean.

Figure 4 - Effect of pH on *A. tenuifolius* seed germination (%).



Table 4 - Effect of pH on starting time of germination, time to 50% germination, mean germination time and germination index of *A. tenuifolius*

pH	T-start ± SE (days)	T-50 ± SE (days)	MGT ± SE (days)	GI ± SE
6.2 (Control)	2.00 d ± 0.00	3.91 d ± 0.05	5.36 f ± 0.17	5.87 a ± 0.11
6.0	3.25 c ± 0.48	5.44 c ± 0.02	6.41 e ± 0.10	4.06 b ± 0.06
6.5	3.50 bc ± 0.50	5.53 bc ± 0.02	7.22 d ± 0.14	3.78 c ± 0.09
7.0	4.00 b ± 0.00	6.77 b ± 0.56	8.23 c ± 0.16	3.39 d ± 0.05
7.5	5.00 a ± 0.00	8.81 a ± 0.76	9.03 a ± 0.14	3.00 e ± 0.01
8.0	5.00 a ± 0.00	8.89a ± 0.12	8.77 ab ± 0.03	2.96 e ± 0.05
8.5	5.00 a ± 0.00	6.76 b ± 0.56	8.67 abc ± 0.10	3.00 e ± 0.04
9.0	5.00 a ± 0.00	6.32 bc ± 0.63	8.37 bc ± 0.36	2.82 e ± 0.08
LSD ≤ 0.05	0.71	1.31	0.51	0.20

Values with same letters did not differ significantly at $P \leq 0.05$. T-start = starting time of germination, T-50 = time to 50% germination, MGT = mean germination time, GI = germination index and SE = standard error.

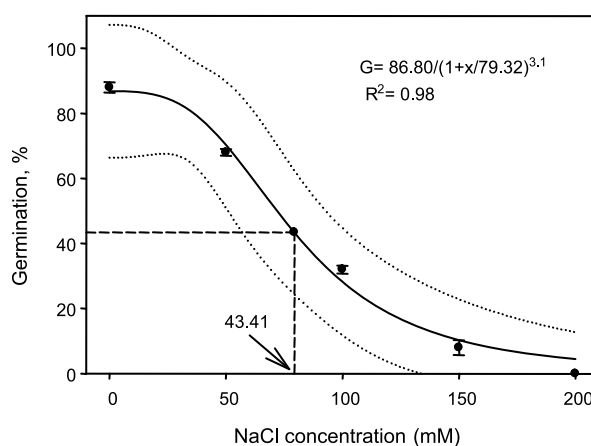
range of pH, under various soil conditions. This behavior of weed plants is very helpful for their successful invasion. Watanabe et al. (2002) reported similar behavior for invasive weed species. Seed germination of many weed species remained unaffected (from pH 4 to 9) in various studies (Thomas et al., 2006; Chachalis et al., 2008; Wang et al., 2009). Germination of coolatai grass was 92% at pH level of 7.00, however at moderate level of pH (5.00) or (9.00) germination was reduced by around 10%. Germination of coolatai grass was reduced at pH level of 4.00 or 10.00 (Chejara et al., 2008). Similar results to those of Chauhan et al. (2006f) were found in *Lolium rigidum* (rigid ryegrass). In contrast, 92 to 95% germination of *Eleusine indica* (goosegrass) was measured at a pH range between 4 and 10 (Chauhan & Johnson, 2008d).

Effect of salt stress

Different salt stress levels exhibited significant effects on *A. tenuifolius* seed germination. It is clear from three parameter logistic model (Figure 4 and 5), that maximum (88%) germination of *A. tenuifolius* was noted with 0 mM (control) and it decreased significantly with a progressive increase in NaCl stress (from 50 to 150 mM) and the lowest (8%) was recorded with 150 mM salt stress. At 200 mM of NaCl stress, germination of *A. tenuifolius* was completely inhibited. A 50% inhibition of *A. tenuifolius* maximum

germination was noted at 80 mM of salt stress (Figure 5).

Starting time of *A. tenuifolius* germination was statistically similar in 0 and 50 mM of NaCl. Greatest starting time of *A. tenuifolius* germination (10 days) was noted at 150 mM of NaCl stress (Table 5). *Asphodelus tenuifolius* also took more days to get 50% germination (11.69 days) and mean germination time (11.42 days) with a NaCl stress level of 150 mM. However, 50 and 100 mM of NaCl stress resulted statistically similar time to get 50%



The bold lines represent a three-parameter logistic model fitted to the data and dotted lines show 95% confidence intervals. Vertical bars represent \pm standard error of the mean. Vertical dash line represents X-axis value at 50% of greatest germination.

Figure 5 - Effect of NaCl concentration (mM) on *A. tenuifolius* seed germination (%).

Table 5 - Effect of salt stress (NaCl) on starting time of germination, time to 50% germination, mean germination time and germination index of *A. tenuifolius*

Salt stress (mM)	T-start ± SE (days)	T-50 ± SE (days)	MGT ± SE (days)	GI ± SE
0 (Control)	2.00 c ± 0.00	5.88 c ± 0.16	6.58 c ± 0.11	4.37 a ± 0.11
50	3.00 c ± 0.00	10.17 b ± 0.06	9.86 b ± 0.03	1.95 b ± 0.08
100	5.75 b ± 0.85	10.63 ab ± 0.30	10.25 b ± 0.45	0.87 c ± 0.08
150	10.0 a ± 0.00	11.69 a ± 0.89	11.42 a ± 0.58	0.17 d ± 0.03
200	N/G	N/G	N/G	N/G
LSD ≤ 0.05	1.32	1.46	1.15	0.25

Values with same letters did not differ significantly at $P \leq 0.05$. N/G non-germinated, T-start = starting time of germination, T-50 = time to 50% germination, MGT = mean germination time, GI = germination index and SE = standard error.

germination and MGT. Lowest time to get 50% germination (5.88 days) and MGT (6.58 days) were recorded with control (0 mM NaCl).

However, with increase in salt stress from 0 to 150 mM, GI of *A. tenuifolius* decreased from 4.37 to 0.17 (Table 5). Our findings suggested that *A. tenuifolius* is very sensitive to salt stress, however a few seeds of *A. tenuifolius* were able to germinate even at high salt stress (150 mM), which enables successful *A. tenuifolius* spread in saline areas around the country. Similar to *A. tenuifolius*, seeds of *Mimosa invisa* (Chauhan & Johnson, 2008a) and *Brassica tournefortii* (Chauhan et al., 2006e) exhibited germination even at very high salt concentration.

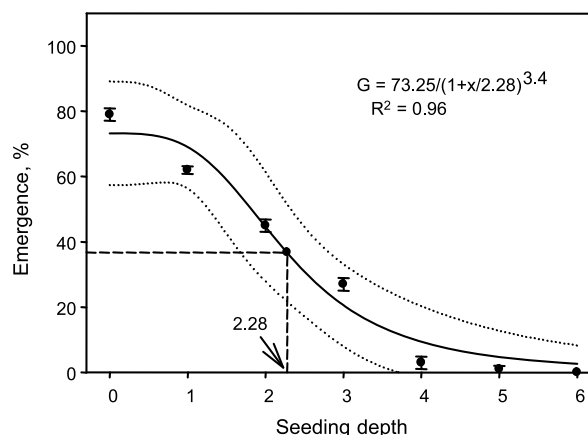
Salts decreased water imbibition by seeds (Bae et al., 2006), inhibiting the germination of many species (Chauhan & Johnson, 2008b). Nandula et al. (2006) found that germination of horse weed decreased with increasing NaCl concentration, and that only 4% germination of horse weed was found at 160 mM NaCl. Similarly, an inverse or negative relationship in seed germination of american sloughgrass (*Beckmannia syzigachne*) (Rao et al., 2008) and *Atriplex verrucifera* (Peng-Peng & Gui-Ling, 2008) was recorded with increased salt concentration.

Effect of seeding depth

Effect of varying seeding depths was significant on *A. tenuifolius* emergence. Greatest emergence of *A. tenuifolius* (79%) was recorded with the seeds placed on soil surface and its emergence was gradually decreased

with increase in seeding depth upto 4 cm (only 3% emergence). Emergence of *A. tenuifolius* was zero at 5/6 cm after 28 days of sowing (Figure 6). Starting time of *A. tenuifolius* emergence was non-significant among all the treatments. Greatest time to get 50% *A. tenuifolius* emergence (9.56 days) and MET (9.52 days) was noted with the seeds placed at 3 cm depth and the lowest time to obtain 50% germination and MET was recorded with *A. tenuifolius* placed at 2 and 1 cm depth (5.06 and 6.20 days, respectively) (Table 6).

Asphodelus tenuifolius showed greatest emergence index with the seeds placed at 1 cm (3.70) and it was statistically similar with



The bold lines represent a three-parameter logistic model fitted to the data and dotted lines show 95% confidence intervals. Vertical dash line represents X-axis value at 50% of greatest germination. Vertical bars represent ± standard error of the mean.

Figure 6 - Effect of seeding depth on *A. tenuifolius* seed emergence (%).



Table 6 - Effect of seeding depth on starting time of emergence, time to 50% emergence, mean emergence time and emergence index of *A. tenuifolius*

Seeding depth (cm)	T-start ± SE (days)	T-50 ± SE (days)	MET ± SE (days)	EI ± SE
0	2.25 ± 0.25	5.88 ab ± 0.16	6.97 ab ± 0.17	3.64 a ± 0.09
1	2.00 ± 0.00	5.10 ab ± 0.16	6.20 ab ± 0.19	3.70 a ± 0.06
2	3.50 ± 0.50	5.06 ab ± 0.36	6.83 ab ± 0.33	2.01 b ± 0.03
3	4.75 ± 0.25	9.56 a ± 0.33	9.52 a ± 0.05	0.82 c ± 0.07
4	4.50 ± 2.63	5.63 ab ± 3.33	5.25 ab ± 3.04	0.07 d ± 0.05
5	N/E	N/E	N/E	N/E
6	N/E	N/E	N/E	N/E
LSD ≤ 0.05	NS	5.00	4.80	0.18

Values with same letters did not differ significantly at $P \leq 0.05$. NS = non-significant, N/E = not-emerged, T-start = starting time of emergence, T-50 = time to 50% emergence, MET = mean emergence time, EI = emergence index and SE = standard error.

soil surface placement (3.64). Emergence index of *A. tenuifolius* decreased progressively after 2 cm depth and lowest was recorded at 4 cm depth (0.07). A 50% inhibition of *A. tenuifolius* greatest emergence occurred at 2.28 cm. Greatest *A. tenuifolius* emergence at soil surface enables its successful distribution and increases its density to economic injury level throughout chickpea producing areas, because practice of no tillage is common in these areas. However, reasonable emergence of *A. tenuifolius* also occurred at 1, 2 and 3 cm burial depths. Studies by Thomas et al. (2006) showed that seeds of *Amaranthus viridis* (Slender Amaranth) emerged in between 0.5 and 2 cm. Contrary to the above findings, the emergence of threehorn bedstraw was negligible at soil surface and it increased with increasing burial depths up to 5 cm (Chauhan et al., 2006d). In contrast, seedling emergence of *Sonchus oleraceus* decreased with increasing seeding depth (Chauhan et al., 2006a).

Results of our laboratory experiments suggested that *A. tenuifolius* can tolerate a wide range of varying environmental factors which is a plus point of *A. tenuifolius* against its management. At 15 °C, germination of *A. tenuifolius* was at its highest; however at 20 and 25 °C considerable *A. tenuifolius* germination was measured. *A. tenuifolius* seeds exhibited greatest germination under light as compared with complete darkness. Increase in water stress significantly decreased *A. tenuifolius* germination. A considerable germination of *A. tenuifolius*

occurred at all pH levels from 6.00 to 9.00, which the pH level of soils in almost all areas of Pakistan. *Asphodelus tenuifolius* is very sensitive to salinity; however a few seeds of *A. tenuifolius* were able to germinate even at 150 mM of salt stress. Seeds of *A. tenuifolius* showed greatest emergence when planted at soil surface, however considerable number of seeds emerged at 1, 2 and 3 cm burial depths. This fact suggests that for a successful management of *A. tenuifolius* in chickpea, a deep burial of its seeds at 5 cm or below is helpful. No tillage practice is common in chickpea growing areas, and that may explain the successful outcome of *A. tenuifolius*.

LITERATURE CITED

- AOSA (ASSOCIATION OF OFFICIAL SEED ANALYSIS). Rules for testing seeds. **J. Seed Technol.**, v. 12, n. 3, p. 1-112, 1990.
- BAE, D. et al. Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. **J. Central Eur. Agric.**, v. 7, n. 2, p. 199-220, 2006.
- BARTLETT MS The use of transformations. **Biometrics.** v. 3, n. 1, p. 39-52. 1947.
- BENVENUTI, S. et al. Germination ecology, emergence and host detection in *Cuscuta campestris*. **Weed Res.**, v. 45, n. 4, p. 270-278, 2005.
- BENVENUTI, S. et al. Germination ecology of *Leptochloa chinensis*: A new weed in the Italian rice agro-environment. **Weed Res.**, v. 44, n. 2, p. 87-96, 2004.

- BHOWMIK P. C. Weed biology: importance to weed management. **Weed Sci.**, v. 45, n. 3, p. 349-356, 1997.
- CHACHALIS, D. et al. Factors affecting seed germination and emergence of venice mallow (*Hibiscus trionum*). **Weed Sci.**, v. 56, n. 4, p. 509-515, 2008.
- CHACHALIS, D.; REDDY, K. N. Factors affecting *Campsis radicans* seed germination and seedling emergence. **Weed Sci.**, v. 48, n. 2, p. 212-216, 2000.
- CHAUHAN, B. S.; JOHNSON, D. E. Seed germination and seedling emergence of synedrella (*Synedrella nodiflora*) in a tropical environment. **Weed Sci.**, v. 57, n. 1, p. 36-42, 2009b.
- CHAUHAN, B. S.; JOHNSON, D. E. Germination ecology of spiny (*Amaranthus spinosus*) and slender amaranth (*A. viridis*): Troublesome weeds of direct-seeded rice. **Weed Sci.**, v. 57, n. 4, p. 379-385, 2009c.
- CHAUHAN, B. S.; JOHNSON, D. E. Seed germination and seedling emergence of giant sensitiveplant (*Mimosa invisa*). **Weed Sci.**, v. 56, n. 2, p. 244-248, 2008a.
- CHAUHAN, B. S.; JOHNSON, D. E. Germination ecology of southern crabgrass (*Digitaria ciliaris*) and indian crabgrass (*Digitaria longiflora*): Two important weeds of rice in tropics. **Weed Sci.**, v. 56, n. 5, p. 722-728, 2008b.
- CHAUHAN, B. S.; JOHNSON, D. E. Influence of environmental factors on seed germination and seedling emergence of eclipta (*Eclipta prostrata*) in a tropical environment. **Weed Sci.**, v. 56, n. 3, p. 383-388, 2008c.
- CHAUHAN, B. S.; JOHNSON, D. E. Germination ecology of goosegrass (*Eleusine indica*): An important grass weed of rainfed rice. **Weed Sci.**, v. 56, n. 5, p. 699-706, 2008d.
- CHAUHAN, B. S.; JOHNSON, D. E. Seed germination ecology of purple-leaf button weed (*Borreria ocymoides*) and indian heliotrope (*Heliotropium indicum*): Two common weeds of rain-fed rice. **Weed Sci.**, v. 56, n. 5, p. 670-675, 2008e.
- CHAUHAN, B. S.; GILL, G.; PRESTON, C. African mustard (*Brassica tournefortii*) germination in southern Australia. **Weed Sci.**, v. 54, n. 5, p. 891-897, 2006c.
- CHAUHAN, B. S. et al. Factors affecting seed germination of annual sowthistle (*Sonchus oleraceus*) in southern Australia. **Weed Sci.**, v. 54, n. 5, p. 854-860, 2006a.
- CHAUHAN, B. S. et al. Seed germination and seedling emergence of threehorn bedstraw (*Galium tricorutum*). **Weed Sci.**, v. 54, n. 5, p. 867-872, 2006d.
- CHEJARA, V. et al. Factors affecting germination of coolatai grass (*Hyparrhenia hirta*). **Weed Sci.**, v. 56, n. 4, p. 543-548, 2008.
- COOLBEAR, P. et al. The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. **J. Exper. Bot.**, v. 35, n. 11, p. 1609-1617, 1984.
- DITOMMASO, A. Germination behavior of common ragweed (*Ambrosia artemisiifolia*) populations across a range of salinities. **Weed Sci.**, v. 52, p. 1002-1009, 2004.
- ELLIS, R. A.; ROBERTS, E. H. The quantification of aging and survival in orthodox seeds. **Seed Sci. Technol.**, v. 9, n. 2, p. 373-409, 1981.
- FRIED, G. et al. Environmental and management factors determining weed species composition and diversity in France. **Agric. Ecos. Environ.**, v. 128, n. 1/2, p. 68-76, 2008.
- GORAI, M. et al. Factors influencing seed germination of medicinal plant *Salvia aegyptiaca* L. (Lamiaceae). **Saudi J. Biol. Sci.**, v. 18, n. 3, p. 255-260, 2011.
- GUMA, I. R. et al. Effect of temperature and salinity on germination of *Salsola vermiculata* L. (chenopodiaceae) from Canary Islands. **J. Arid Environ.**, v. 74, n. 6, p. 708-711, 2010.
- HARRINGTON, T. B. Seed germination and seedling emergence of scotch broom (*Cytisus scoparius*). **Weed Sci.**, v. 57, n. 6, p. 620-626, 2009.
- HARRISON, S. et al. Seed size and burial effects on giant ragweed (*Ambrosia trifida*) emergence and seed demise. **Weed Sci.**, v. 55, n. 1, p. 16-22, 2007.
- HOLM, R. et al. World weeds, Natural Histories and distribution. New York: John Wiley and sons, 1977. p. 86.
- INGOLIA, M. et al. Germination ecology of *Rorippa subumbellata* (Tahoe yellow cress), an endangered, endemic species of Lake Tahoe. **Seed Sci. Technol.**, v. 36, n. 3, p. 621-632, 2008.
- KABAT, S. M. et al. Temperature and light affects germination ecology of commercially produced seeds of leavenworth's coreopsis. **Native Plants J.**, v. 8, n. 3, p. 236-247, 2007.
- KOGER, C. H. et al. Factors affecting seed germination, seedling emergence and survival of texasweed (*Caperonia palustris*). **Weed Sci.**, v. 52, n. 6, p. 989-995, 2004.
- LU, P. et al. Effects of environmental factors on germination and emergence of crofton weed (*Eupatorium adenophorum*). **Weed Sci.**, v. 54, n. 3, p. 452-457, 2006.
- MALIK, M. S. et al. Temperature and light requirements for wild radish (*Raphanus raphanistrum*) germination over a 12-month period following maturation. **Weed Sci.**, v. 58, n. 2, p. 136-140, 2010.



- MARTINKOVA, Z. et al. Seed age and storage conditions influence germination of barnyardgrass (*Echinochloa crus-galli*). **Weed Sci.**, v. 54, n. 2, p. 298-304, 2006.
- MASIN, R. et al. Temperature and water potential as parameters for modeling weed emergence in central-northern Italy. **Weed Sci.**, v. 58, n. 3, p. 216-222, 2010.
- MENNAN, H.; NGOUAJIO, M. Seasonal cycles in germination and seedling emergence of summer and winter populations of catchweed bedstraw (*Galium aparine*) and wild mustard (*Brassica kaber*). **Weed Sci.**, v. 54, n. 1, p. 114-120, 2006.
- MICHEL, B. E.; KAUFAMAN. M. R. The osmotic potential of polyethylene glycol 6000. **Plant Physiol.**, v. 51, n. 5, p. 914-916, 1973.
- NANDULA, V. K. et al. Factors affecting germination of horseweed (*Conyza canadensis*). **Weed Sci.**, v. 54, n. 5, p. 898-902, 2006.
- NASIR, Z. A.; SULTAN. S. Survey of weeds in mustard fields of district Chakwal, Pakistan. **Pakistan J. Biol. Sci.**, v. 7, n. 2, p. 279-286, 2004.
- NEMATI, I. et al. The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (*Oryza sativa* L.) seedlings. **Plant Soil Environ.**, v. 57, n. 1, p. 26-33, 2011.
- NORSWORTHY, J.; OLIVEIRA. M. Sicklepod (*Senna obtusifolia*) germination and emergence as affected by environmental factors and seeding depth. **Weed Sci.**, v. 54, n. 5, p. 903-909, 2006.
- PAHLEVANI, A. H. et al. Effects of environmental factors on germination and emergence of swallowwort. **Weed Technol.**, v. 22, n. 2, p. 303-308, 2008.
- PENG-PENG, L.; GUI-LING. Z. Germination and seedling growth of *Atriplex verrucifera* Bieb. Under NaCl stress. **Acta Bot. Boreali-Occidentalia Sinica**, v. 28, n. 12, p. 12, 2008.
- PONIA, B. L. et al. Weed management in wheat (*Triticum aestivum*) with special reference to *Asphodelus tenuifolius*. **Indian J. Weed Sci.**, v. 33, n. 3-4, p. 100-103, 2001.
- RAO, N. et al. Influence of environmental factors on seed germination and seedling emergence of american sloughgrass (*Beckmannia syzigachne*). **Weed Sci.**, v. 56, n. 4, p. 529-533, 2008.
- SAS. Statistical Analysis Systems. **SAS Procedures Guide**. Version 9.0, Cary: Statistical Analysis Systems Institute, 2002.
- STEEL, R. G. D. et al. **Principles and procedures of statistics**. Multiple comparisons. 3.ed. New York: McGraw Hill Book, 1997. p. 178-198.
- THOMAS, W. et al. Influence of environmental factors on slender amaranth (*Amaranthus viridis*) germination. **Weed Sci.**, v. 54, n. 2, p. 316-320, 2006.
- TIWARI, A. N. et al. Crop-weed competition studies in chickpea having *Asphodelus tenuifolius* dominated weed community under rain fed condition. **Indian J. Weed Sci.**, v. 33, n. 3&4, p. 198-199, 2001.
- WANG, J. J. et al. Factors affecting seed germination of cadillo (*Urena lobata*). **Weed Sci.**, v. 57, n. 1, p. 31-35, 2009.
- WATANABE, H. et al. Environmental factors affecting germination of apple of Peru. **Weed Sci.**, v. 50, n. 2, p. 152-156, 2002.
- WIDDERICK, M. J. et al. Better management of *Sonchus oleraceus* L. (common sowthistle) based on the weed's ecology. In: AUSTRALIAN WEEDS CONFERENCE, 14., 2004, Wagga. **Proceedings...** Wagga: New South Wales, Weed Society of New South Wales, 2004. p.535-537.
- ZHOU, J. et al. Factors affecting germination of hairy nightshade (*Solanum sarrachoides*) seeds. **Weed Sci.**, v. 53, n. 1, p. 41-45, 2005.

