Research Article

Germination responses of vipergrass (*Dinebra retroflexa*) to environmental factors and herbicide options for its control

Sachin Dhanda[1], Kartik Sharma[2] and Bhagirath Singh Chauhan[3]

[1]Graduate Research Assistant, Department of Agronomy, Kansas State University, Manhattan, KS, USA; [2]Doctoral Student, Department of Agronomy, Punjab Agricultural University, Ludhiana, Punjab, India and [3]Professor, Queensland Alliance for Agriculture and Food Innovation (QAAFI) and School of Agricultural and Food Sciences (SAFS), University of Queensland, Brisbane, Queensland, Australia; Adjunct Professor, CCSHAU, Hisar, Haryana, India

Abstract

Vipergrass [*Dinebra retroflexa* (Vahl) Panzer] is an annual weed of the Poaceae family distributed in several parts of Australia, Asia, and Europe. Very limited information is available on its germination response to different environmental conditions. Knowledge of its seed ecology and biology could help in formulating better weed management decisions. Experiments were conducted to study the effect of alternating temperatures, light conditions, salt stress, water stress, seed burial depths, and wheat residue amounts on the germination or emergence of *D. retroflexa*. Also, different pre- and postemergence herbicides were evaluated to control *D. retroflexa*. The highest germination (98%) was recorded at 30/20 C followed by 35/25 C (95%). Light was required for the germination of *D. retroflexa*. Germination decreased with an increase in sodium chloride (NaCl) concentrations. Even at 80 mM NaCl, 81% of seeds germinated, indicating *D. retroflexa*'s high salt tolerance. Seed germination gradually decreased with an increase in water stress, and no germination was recorded at −0.8 MPa osmotic potential. The emergence of *D. retroflexa* decreased with an increase in seed burial depths. The highest germination (83%) was recorded for surface-sown seeds, and emergence was reduced to 0 at a burial depth of 2 cm. Seedling emergence decreased from 82% to 2% when the crop residue load was increased from 0 to 800 kg ha^{-1}. Applications of preemergence herbicides (at field rates), such as diuron, isoxaflutole, pendimethalin, pyroxasulfone, *S*-metolachlor, terbuthylazine, and triallate, and postemergence herbicides, such as clethodim, haloxyfop-methyl, glufosinate, glyphosate, imazamox plus imazapyr (a commercial mixture), and paraquat, resulted in complete control (100%) of *D. retroflexa*. Knowledge gained from this study will help us to understand the potential spread of *D. retroflexa* to other areas and to formulate integrated weed management strategies for its effective control.

Introduction

Weeds are one of the major biotic factors in reducing crop yield. In Australia, it is estimated that weeds cause a crop yield loss of around 2.8 billion kg of grain yr^{-1} and cost grain producers more than AU\$3.3 billion yr^{-1} (Llewellyn et al. 2016). Vipergrass [*Dinebra retroflexa* (Vahl) Panzer] is an annual grass belonging to the Poaceae family. The genus *Dinebra* is closely related to the *Leptochloa* genus (Snow and Peterson 2012). *Dinebra retroflexa* is native to Africa and is widely distributed throughout Asia, Europe, and Australia (CABI 2022; Munirathnam and Kumar 2014; Nayak and Satapathy 2015). It is a major weed in many field crops, including sugarcane (*Saccharum officinarum* L.), cotton (*Gossypium hirsutum* L.), peanut (*Arachis hypogaea* L.), sorghum [*Sorghum bicolor* (L.) Moench], onion (*Allium cepa* L.), and rice (*Oryza sativa* L.) (Babiker and Ahmed 1986; Ghobrial 1981; Ibrahim 1984; Karar et al. 2005). In a survey conducted in the cotton region of Australia, this weed was present in 4% of the fields surveyed, indicating the potential for this species to evolve resistance to glyphosate in glyphosate-resistant cotton (Manalil et al. 2017).

Dinebra retroflexa is a 15- to 112-cm-tall grass. The ligule is membranous and laciniate at the apex. The inflorescence is composed of many linear racemes, distributed along the floral axis (Anonymous 2022; CABI 2022; Satyamurty 1985). Each raceme is composed of 2 to 20 fertile spikelets that are sessile and compressed with 2 or 3 flowers. *Dinebra retroflexa* is characterized by its reddish anthers and ellipsoid-shaped caryopsis (Anonymous 2022; CABI 2022; Satyamurty 1985). It multiplies through seeds, and during the dissemination, the racemes detach themselves completely from the floral axis (Tanji 2020). Knowledge of the physiological response of weeds to the environment is important to understand how they interact with crops under field conditions (Chauhan and Johnson 2010). Studies related to weeds' interactions with

different environmental factors help in managing weeds. For example, if a weed species needs light to germinate, then creating dark conditions with residue or mulch can help in reducing the weed seedbank.

Germination is the initial phase in the life cycle of any plant. Temperature is one of the most important abiotic factors having a direct impact on physiological and metabolic processes by affecting moisture uptake and thus altering the germination behavior (Bewley et al. 2013). Along with temperature, other environmental factors such as light, moisture, and soil salinity can influence the germination of many weed species (Mahajan and Chauhan 2020; Mahajan et al. 2018; Singh et al. 2021). Seed burial depth and crop residue on the soil surface could affect weed seedling emergence (Mahajan et al. 2021). Chauhan and Johnson (2008) found that seedling emergence of goosegrass [*Eleusine indica* (L.) Gaertn.] was highest (82%) for seeds placed on the soil surface, but decreased exponentially with an increasing burial depth, and no seedlings emerged from a burial depth of 8 cm.

In Australia, about 3% of the total soil area is affected by salinity, which reduces the productivity of crops and pastures grown on those soils (Bot et al. 2000). This study provides information regarding the spread of *D. retroflexa* under saline soil conditions. A better understanding of the effect of environmental factors on the seed germination of *D. retroflexa* will help in devising the best management practices for its control. Limited literature is available on the germination behavior of *D. retroflexa* under different environmental regimes. However, in Sudan, Hamada et al. (1993) studied the effect of temperature, light, and drought on the germination of several weed species, including *D. retroflexa*, but information on the effect of salinity stress, burial depth, and residue retention on *D. retroflexa* germination/emergence is lacking. Therefore, this study was conducted to evaluate the effects of alternating day/night temperature, light, salt stress, water stress, burial depth, and residue retention on seed germination and seedling emergence of *D. retroflexa*.

Herbicides are an important tool in many integrated weed management (IWM) programs. Very limited information is available on pre- and postemergence herbicide options to control *D. retroflexa*. This study also evaluated the response of *D. retroflexa* to commonly used pre- and postemergence herbicides. Information on effective herbicides can be used to control existing infestations of *D. retroflexa*.

Materials and Methods

Seed Collection

Seeds used in this study were originally collected from Gatton Farms, University of Queensland, Australia, in April 2021 (GPS coordinates: 27.3245°S, 152.1944°E). Seeds were collected from 50 to 60 randomly selected plants. After collection, seeds were stored in paper bags at room temperature (25 C) until the germination test.

General Protocol

All germination experiments were conducted in the weed science laboratory of the Queensland Alliance for Agriculture and Food Innovation (QAAFI) at the University of Queensland, Gatton, Australia. Petri dishes (Rowe Scientific Pty Ltd, Brisbane, QLD, Australia; 9-cm diameter) containing a bilayer of Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, Kent, UK) were used for all germination experiments. About 98% of

seeds germinated in a preliminary test, and the "seed pressing technique" showed that the rest of the seeds were viable. Before placement of seeds, filter papers were moistened using 5 ml deionized water, polyethylene glycol (PEG), or sodium chloride (NaCl) solutions with the aid of a micropipette (BOECO, Hamburg, Germany). Germination tests were performed by placing 25 seeds evenly in a petri dish with a pair of forceps and a desk magnifying lamp. Uniformly sized seeds were selected, and diseased, damaged, and unevenly shaped seeds were discarded.

All petri dishes were kept in plastic ziplock bags to minimize evaporation losses, and the bags were placed under temperature- and light intensity–controlled incubators (Labec Laboratory, Sydney, NSW, Australia). A white light with an intensity of 85 mol m^{-2} s^{-1} was provided by fluorescent lamps (Ultralamp ECO-T5, Cabramatta, NSW, Australia) installed inside the incubator. Except for the temperature experiment, all other experiments in the incubator were configured at a temperature range of 30/20 C and an alternating photoperiod of 12-h/12-h light and dark. This temperature range was found to be the optimum temperature regime, and no further germination was observed after 42 d at this temperature regime (30/20 C) in the temperature experiment. Therefore, all experiments were terminated after 42 d, except the temperature experiment, which ran for 70 d.

Experiment 1. Effect of Temperature on Germination

Incubators were calibrated to five different day/night temperature regimes, that is, 15/5, 20/10, 25/15, 30/20, and 35/25 C, with 12-h-light and 12-h-dark environments. Petri dishes with seeds of *D. retroflexa* were placed in the incubator maintained at the five alternating day/night temperature regimes, and the effect on *D. retroflexa* germination was evaluated. The selected five temperature treatments represent the prevailing temperature conditions in Australia during different cropping seasons. Germination was assessed after every 7 d for 70 d.

Experiment 2. Effect of Light on Germination

Dinebra retroflexa seeds were exposed to alternating light (12-h light/12-h dark) and dark (24-h dark) environments to evaluate the effect of different photoperiods on seed germination. The light photoperiod in the incubator coincided with the high temperature (30 C) and the alternative dark photoperiod corresponded with the low temperature (20 C). Petri dishes were wrapped with aluminum foil (trilayer) to achieve completely dark conditions and allowed to incubate for 42 d before assessment of germination data.

Experiment 3. Effect of NaCl on Germination

An experiment to evaluate the effect of salt stress on the germination of *D. retroflexa* was established using NaCl concentrations of 0, 20, 40, 80, 160, and 320 mM. The general seed germination protocol was followed for seed incubation, and filter papers were moistened with 5 ml of salt solutions according to the treatments. The required concentrations were prepared using NaCl (Rowe Scientific Pty Ltd) and deionized water. These different concentrations represent the extent of salinity levels in different parts of Australia. Germination was assessed for 42 d at 7-d intervals.

Experiment 4. Effect of Osmotic Potential on Germination

To assess the effect of water potential on germination of *D. retroflexa*, seeds were placed in the incubator under different water potentials, that is, 0 (control), −0.1, −0.2, −0.4, −0.8, and

−1.6 MPa. Solutions of desired osmotic potentials were prepared using PEG 8000 (Sigma-Aldrich, St Louis, MO, USA) following the procedure described by Michel and Radcliffe (1995). Germination was assessed for 42 d at 7-d intervals.

Experiment 5. Effect of Seed Burial Depth on Emergence

An experiment to study the effect of burial depth on the emergence of *D. retroflexa* was established using plastic pots (14-cm diameter) filled with the field soil. The soil was collected from Gatton Farms of the University of Queensland and sieved through a 3-mm sieve before pots were filled. The soil texture was clay loam with 35% sand, 38% silt, and 28% clay with 2.7% organic matter. The soil was neutral with a pH of 7.1. A total of 50 seeds per pot were placed either on the soil surface (0-cm depth) or at different soil depths (0.5, 1.0, 2.0, and 4.0 cm). The pots were kept in nonporous plastic trays full of water before being transferred to the incubator. The incubator was calibrated at day/night temperatures of 30/20 C with 12-h light/12-h dark. On every third day, water was added manually to the plastic trays to maintain an optimum level of moisture in the pots. Emergence counts were taken at 7-d intervals for 42 days.

Experiment 6. Effect of Wheat Crop Residue Amount on Emergence

To study the effect of wheat (*Triticum aestivum* L.) crop residue amount on the emergence of *D. retroflexa*, 50 seeds were sown on the surface (0-cm depth) in plastic pots (14-cm diameter). The same soil was used to fill the pots as described earlier. After seed sowing, chopped air-dried wheat ('Spitfire') residue was added to the surface at amounts equivalent to 0, 1,000, 2,000, 4,000, and 8,000 kg ha^{-1}. The pots were kept in nonporous plastic trays filled with water before being transferred to the incubator maintained at an alternating day/night temperature of 30/20 C. Throughout the experiment, the soil was kept under moist conditions through the manual addition of water to the plastic trays on every third day. Seedling emergence counts were taken at 7-d intervals for 42.

Experiment 7. Effect of Pre- and Postemergence Herbicides on Survival and Biomass

Treatment details for pre- and postemergence herbicides are given in Table 1. To study the effect of preemergence herbicides, 30 seeds were uniformly planted on the soil surface in pots (14-cm diameter) and then covered with a 0.2-cm layer of soil. The same soil was used to fill the pots as described previously. All preemergence herbicides were sprayed immediately after sowing using a research track sprayer equipped with TeeJet® XR 110015 (TeeJet, Glendale Heights, IL, USA; Sprayshop, Toowoomba, QLD, Australia) flat-fan nozzles calibrated to an output spray volume of 108 L ha^{-1} and spray pressure of 140 kPa. Pots were kept dry until 24 h after spraying and were watered thereafter using a sprinkler system. A control treatment was included in which no herbicide was sprayed. At 28 d after treatment (DAT), plant survival was recorded and aboveground biomass was harvested for the surviving plants and oven-dried for 72 h at 70 C to determine dry biomass.

To evaluate the efficacy of postemergence herbicides, 12 seeds of *D. retroflexa* were planted in pots (14-cm diameter). After emergence, thinning was done to maintain 5 plants per pot. Herbicides were sprayed at two stages of *D. retroflexa*, that is, the 5- to 6- and 11- to 12-leaf stages, using the same research track sprayer as described earlier. At 28 DAT, survival percentage and biomass were recorded as described earlier.

Table 1. Treatment details for pre- and postemergence herbicides.

Treatment	Rate g ai ha^{-1}	Adjuvant
Preemergence herbicides		
Control	—	—
Dimethanamid-P	720	—
Diuron	1,800	—
Imazethapyr	70	—
Isoxaflutole	75	—
Pendimethalin	880	—
Pyroxasulfone	100.3	—
S-metolachlor	1,920	—
S-metolachlor + prosulfocarb	2,300	—
Terbuthylazine	1,050	—
Triallate	1,600	—
Trifluralin	960	—
Postemergence herbicides		
Control	—	—
Clethodim	120	1% Cando[TMa]
Haloxyfop	78	1% Hasten[TMb]
Glufosinate	750	—
Glyphosate	570	—
Imazamox + imazapyr	36	1% Hasten[TM]
Paraquat	600	1% Hasten[TM]

[a]Nufarm, Australia Ltd, Laverton North, VIC, Australia.
[b]BASF Australia Ltd, Southbank, VIC, Australia.

Statistical Analyses

Petri dishes were the experimental units for experiments 1 to 4, while pots were the experimental units for experiments 5 to 7. All experiments were conducted in a randomized complete block design, and each treatment was replicated three times. Each replication was arranged on a different shelf in the incubator and considered a block. Each experiment was repeated over time. No interaction was observed between the experimental runs and treatments in each experiment; therefore, the data from both runs were pooled for further analysis (Genstat 2021). Before analysis, data were visually examined for homogeneity of the variance by plotting residuals (Genstat 2021). Data were analyzed using ANOVA, and graphs were plotted using SigmaPlot v. 14.5 Notebook (Systat Software, San Jose, CA, USA).

A three-parameter sigmoid model was fit to germination or emergence data obtained at different times (*d*) in temperature, salt stress, osmotic potential, burial depth, and wheat residue retention experiments. The model was:

$$G = a/1 + e[-(x - T_{50})/b] \qquad [1]$$

where *G* is cumulative germination (%) at time *x*, *a* is maximum germination (%), T_{50} is the time (in days) required for 50% of maximum germination, and *b* is the slope. An exponential decay model was fit to the final seedling emergence (%) as influenced by different seed burial depths. The model was:

$$E = a * e^{-bD} \qquad [2]$$

where *E* is emergence (%) at burial depth *D*, *a* is the maximum emergence, and *b* is the slope.

The following linear model was fit to the final emergence (%) obtained at different residue amounts:

$$E = a - bR \qquad [3]$$

where *E* is the emergence (%) at crop residue amount *R*, *a* is the maximum emergence, and *b* is the slope. The fitness of the selected

models was determined using R^2 values. The parameter estimates were compared using their standard errors. Light and herbicide experiments were analyzed using ANOVA (Genstat 2021). The treatment means were separated using Fisher's protected LSD test at a 5% level of significance.

Results and Discussion

Experiment 1. Effect of Temperature on Germination

Temperature regimes significantly affected the seed germination of *D. retroflexa*. The highest germination (98%) was recorded at 30/20 C day/night temperature, and germination decreased at temperatures lower and higher than 30/20 C (Figure 1; Table 2). The germination of *D. retroflexa* decreased rapidly at lower temperature regimes, and no seeds germinated at 15/5 C. It took 5 d to achieve 50% germination at 30/20 C and 35/25 C, whereas 42 d were required to reach 50% germination at 20/10 C.

Similar results have been reported in a study conducted in Sudan, where the highest germination of *D. retroflexa* was found at 30/20 C in alternating 12-h/12-h light and dark conditions (Hamada et al. 1993). Mucronate sprangletop [*Leptochloa panicea* (Retz.) Ohwi; syn.: *Dinebra panicea* var. *brachiata* (Steud.) P.M. Peterson & N. Snow], a closely related species, has been reported to have maximum germination at temperatures ranging from 25/15 to 30/20 C (Weller et al. 2019). Other weed species similar to *D. retroflexa*, including tighthead sprangletop [*Leptochloa panicoides* (J. Presl) Hitchc.], green sprangletop [*Leptochloa dubia* (Kunth) Nees], kallar grass [*Leptochloa fusca* (L.) Kunth], bearded sprangletop [*Leptochloa fusca* (L.) Kunth ssp. *fascicularis* (Lam.) N. Snow], and Chinese sprangletop [*Leptochloa chinensis* (L.) Nees], were also found to have a maximum germination between 20 to 35 C (Altop et al. 2015; Baskin et al. 1999; Benvenuti et al. 2003; Chauhan and Johnson 2008; Roundy and Biedenbender 1996).

Results from this experiment suggest that *D. retroflexa* can germinate at a broad range of temperatures, that is, in summer, spring, and autumn seasons in northern Australia. Although seeds cannot germinate in cold temperatures (15/5 C), they can germinate as the temperature increases up to 30/20 C, but beyond this, germination will again decline. This indicates that extreme cold and hot environmental conditions are not favorable for the germination of *D. retroflexa*, and thus it might be a less problematic weed under these environmental conditions. Further, seeds may be dormant under such conditions, waiting for optimal conditions to germinate. *Dinebra retroflexa*'s potential to germinate in summer, spring, and autumn seasons in northern Australia (Queensland and New South Wales) will create a challenge in managing this weed in fallow and crops, such as sorghum and cotton.

Experiment 2. Effect of Light on Germination

The germination of *D. retroflexa* was 98% in the alternating light/dark (12 h/12 h) conditions at 30/20 C, while only 1% of seeds germinated under complete-dark (24 h) conditions. When the ungerminated seeds from the dark environment were introduced back to light conditions (12-h light/12-h dark), germination increased to 98%. These results showed that light plays an important role in the germination of *D. retroflexa*. These results are in line with a previous study, in which only 2% of *D. retroflexa* seeds germinated under complete-dark conditions (Hamada et al. 1993). Further, those authors reported 97% germination under light conditions (12-h/12-h light/dark), indicating the significant effect of light conditions on the germination of *D. retroflexa*. *Leptochloa*

Table 2. Effect of alternating day/night temperature regimes (15/5 to 35/25 C) on germination of *Dinebra retroflexa*.[a]

| Temperature regime | Parameter estimates (±SE) | | | R^2 |
	a	b	T_{50}	
C	%		d	
15/5		0% germination		
20/10	41.8 (2.8)	8.7 (1.5)	41.9 (2.0)	0.98
25/15	55.8 (2.2)	10.8 (1.4)	25.3 (1.7)	0.98
30/20	98.4 (0.3)	0.5 (14.1)	5.3 (0.5)	0.99
35/25	95.3 (0.9)	0.5 (18.6)	5.3 (0.6)	0.99

[a]A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the germination data. In the equation, G represents the cumulative germination percentage at time x, a is the maximum germination percentage, T_{50} is the time (in days) required for 50% of maximum germination, and b is the slope.
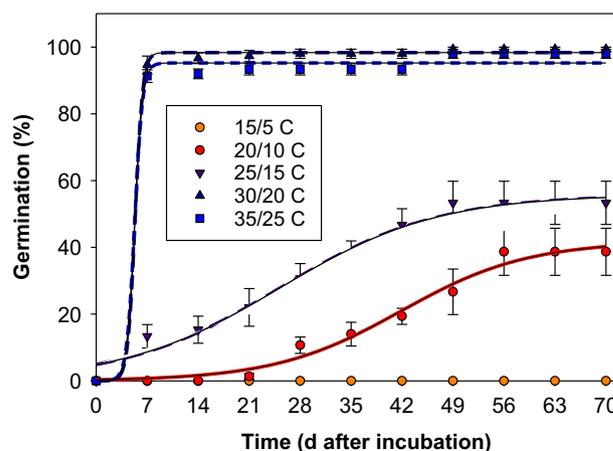

Figure 1. Effect of alternating day/night temperature regimes (15/5 to 35/25 C) on germination of *Dinebra retroflexa*. The vertical bars represent the standard errors of the means. A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the germination data; parameter estimates are given in Table 2.

chinensis, a related weed species, was also found to exhibit photoblastic behavior, with germination completely inhibited under dark conditions (Benvenuti et al. 2003; Chauhan and Johnson 2008). However, in *L. panicea*, 43% germination has been reported in complete darkness at 35/20 C (Baskin et al. 1999). This differential germination response might be due to genetic differences among species in different agroecological conditions.

The results from this experiment showed that the seeds of *D. retroflexa* are highly photoblastic in nature, which means they have a specific light requirement for germination. This indicates *D. retroflexa*'s potential to become a more problematic weed under zero-till conditions (Cousens et al. 1993), because weed seeds remain on or close to the soil surface under these systems. This information can be used to reduce the germination of *D. retroflexa* by providing shade on the ground or covering the ground with a mulch. Further, shallow tillage may help in reducing its germination by burying seeds in the soil at shallow depths.

Experiment 3. Effect of NaCl on Germination

Salt stress had a substantial impact on *D. retroflexa* germination. The highest germination (98%) was recorded at 0 mM NaCl (no-stress conditions), and germination gradually decreased as NaCl concentration successively increased (Figure 2; Table 3). However, 81% of seeds germinated even at 80 mM NaCl, indicating

Table 3. Effect of sodium chloride (NaCl) concentrations on seed germination of *Dinebra retroflexa*.[a]

NaCl concentration	Parameter estimates (±SE)			R^2
	a	b	T_{50}	
mM	%		d	
0	97.6 (0.6)	0.7 (1.9)	5.6 (3.7)	0.99
20	93.7 (1.4)	0.8 (2.4)	5.5 (4.2)	0.99
40	87.0 (1.9)	1.4 (0.9)	5.9 (0.7)	0.99
80	80.8 (3.7)	3.9 (1.1)	8.2 (1.2)	0.97
160	12% germination (curve did not fit)			
320	0% germination			

[a]A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the germination data. In the equation, G represents the cumulative germination percentage at time x, a is the maximum germination percentage, T_{50} is the time (in days) required for 50% of maximum germination, and b is the slope.

Table 4. Effect of osmotic potential on germination of *Dinebra retroflexa*.[a]

Osmotic potential	Parameter estimates (±SE)			R^2
	a	b	T_{50}	
MPa	%		d	
0	93.1 (2.4)	1.0 (2.0)	5.6 (2.8)	0.98
−0.1	78.3 (1.9)	0.8 (5.7)	5.6 (10.0)	0.99
−0.2	68.0 (3.4)	1.1 (2.8)	5.3 (4.5)	0.94
−0.4	25.4 (0.3)	1.8 (0.2)	12.2 (0.3)	0.99
−0.8	0% germination			
−1.6	0% germination			

[a]A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the germination data. In the equation, G represents the cumulative germination percentage at time x, a is the maximum germination percentage, T_{50} is the time (in days) required for 50% of maximum germination, and b is the slope.
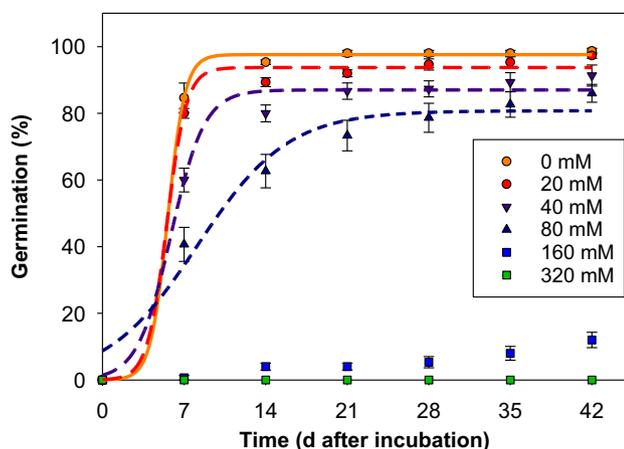
Figure 2. Effect of sodium chloride (NaCl) concentrations on germination of *Dinebra retroflexa*. The vertical bars represent the standard errors of the means. A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the germination data; parameter estimates are given in Table 3.

Figure 3. Effect of osmotic potential on germination of *Dinebra retroflexa*. The vertical bars represent the standard errors of the means. A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the germination data; parameter estimates are given in Table 4.
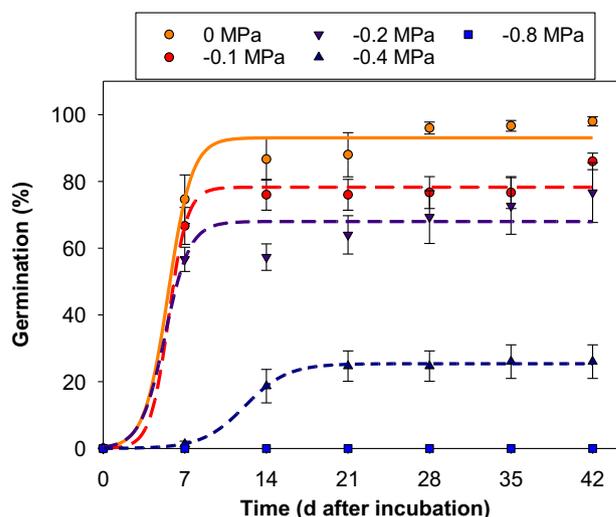
high salt tolerance for germination in this weed species. Germination declined rapidly at more than 80 mM NaCl, and only 12% of seeds germinated at 160 mM NaCl. No seeds germinated at 320 mM NaCl. The time required for 50% germination was increased from 5 to 8 d with an increase in the NaCl concentration from 0 to 80 mM.

Weller et al. (2019) also found greater than 80% germination in a closely related species, *D. panicea* var. *brachiata*, at a 100 mM NaCl concentration. Similarly, Altop et al. (2015) also reported that germination of *L. fusca* ssp. *fascicularis* declined with an increase in NaCl concentrations from 0 to 100 mM. About 58% germination was observed at 100 mM NaCl, but no germination occurred at 400 mM NaCl. Another summer grass species, feather fingergrass (*Chloris virgata* Sw.), has been reported to be more salt sensitive, as 50% germination was recorded at 25 mM NaCl (Fernando et al. 2016). A high salt concentration inhibits seed germination due to physiological disturbance of metabolic processes that are affected by ion toxicity (Farooq et al. 2015). Further, a high concentration of salt creates a low osmotic potential around the seed, which prevents water uptake and thus inhibits germination (Welbaum et al. 1990). Soils having a NaCl concentration of 20 mM are characterized as salt-affected (Abrol et al. 1988). The results of this experiment indicate that *D. retroflexa* is a salt-tolerant weed species, and due to the increased area with

salt-affected soils in the dry region of Australia (Rengasamy 2002), *D. retroflexa* could soon be a concern in these areas.

Experiment 4. Effect of Osmotic Potential on Germination

Osmotic potential was observed to have a significant effect on the germination of *D. retroflexa*. At 0 MPa, the maximum seed germination (93%) was recorded, and germination decreased with an increase in water stress (Figure 3; Table 4). Only 25% germination was observed at −0.4 MPa, and no germination occurred at −0.8 MPa and −1.6 MPa. It took about 6 d for 50% germination at 0 MPa, while it took double that time (12 d) to reach 50% germination at −0.4 MPa.

Similar to the current study, Hamada et al. (1993) also reported a decreasing trend of germination of *D. retroflexa* with increasing water stress. Likewise, 97% germination was reported in *D. panicea* var. *brachiata* under no moisture stress (0 MPa), and germination was reduced to 55% at −0.8 MPa (Weller et al. 2019). In contrast to the gradual decline in the germination of *D. retroflexa*, some grass species are found to be more sensitive to water stress. *Chloris virgata*, for example, showed a 50% reduction in germination with a slight change in osmotic potential from 0 MPa to −0.09 MPa

(Fernando et al. 2016). The results of the current study indicate high germination of *D. retroflexa* under irrigated conditions, but it can also tolerate water stress to some extent, as almost 70% of seeds germinated even at −0.2 MPa, demonstrating water-stress tolerance. Therefore, *D. retroflexa* could be a problem for growers to manage under salt- and moisture-stress conditions. Australia regularly faces drought conditions that impact crop production. Results suggest that *D. retroflexa* will further impact crop production under such conditions.

Experiment 5. Effect of Seed Burial Depth on Emergence

A significant effect of seed burial depth was observed on the seedling emergence of *D. retroflexa*. The highest germination (83%) was recorded for seeds placed on the soil surface (0 cm), and emergence decreased to 13% and 9% at seed burial depths of 0.5 and 1.0 cm, respectively (Figure 4; Table 5). A burial depth of 0.2 cm was required to inhibit 50% of seedling emergence. The time required to accomplish 50% emergence from 0, 0.5, and 1.0 cm was almost similar (10 to 11 d). At 2-cm and greater depths, seedling emergence was completely inhibited. Weller et al. (2019) also found the highest germination (50%) of *D. panicea* var. *brachiata* on the soil surface. Previous research also reported that more than 80% of seeds of *L. chinensis* germinated on the soil surface, and emergence occurred only from depths shallower than 2 cm (Benvenuti et al. 2003; Chauhan and Johnson 2008).

Other small-seeded species, such as feather lovegrass [*Eragrostis tenella* (L.) P. Beauv. ex Roem. & Schult.], also showed the highest germination (69%) from the soil surface (Chauhan 2013). *Dinebra retroflexa* seeds are photoblastic, as made evident by the light/dark experiment, thus explaining greater germination on the soil surface compared with burial at varying depths. Furthermore, anaerobic soil conditions and limited food reserves are possible reasons for low germination or emergence from deep seed burial depths (Baskin and Baskin 1998). The high germination of *D. retroflexa* on the soil surface demonstrates its potential to establish under no-till conditions, where most of the seeds remain on the soil surface (Chauhan et al. 2006). Therefore, a shallow-tillage operation could help in burying the seeds of *D. retroflexa* below the depth of emergence.

Experiment 6. Effect of Wheat Crop Residue Amount on Emergence

Seedling emergence of *D. retroflexa* was significantly affected by the amount of wheat residue on the soil surface. The highest emergence (82%) was recorded under a no-residue load (Figure 5; Table 6). Emergence was reduced from 72% to 28% with an increase in the residue load from 1,000 to 4,000 kg ha^{-1}, and only 2% emergence was recorded under a residue load of 8,000 kg ha^{-1}. A residue load of 3,900 kg ha^{-1} was required to reduce emergence by 50%. The time required to reach 50% emergence increased from 7 to 13 d with the increase in the residue load from 0 to 4,000 kg ha^{-1}. The high-residue amount may have created dark conditions for seeds, and thus the lack of light and the physical barrier created due to the residue load might be the reasons for the poor emergence of *D. retroflexa*.

Another summer grass species, windmillgrass (*Chloris truncata* R. Br.), has been reported to have the greatest emergence (67%) under no-residue conditions, and emergence declined exponentially with an increase in sorghum residue amount. Only 7% to 15% emergence was recorded with a residue of 4,000 to 6,000 kg ha^{-1}

Table 5. Effect of seed burial depth on emergence of *Dinebra retroflexa*.[a]

| Burial depth | Parameter estimates (±SE) | | | R² |
	a	b	T₅₀	
cm	%		d	
0	83.3 (1.7)	2.8 (0.4)	11.4 (0.5)	0.99
0.5	13.3	0.12	10.5	0.99
1.0	9.3	0.12	10.4	0.99
2.0	0% emergence			
4.0	0% emergence			

[a]A three-parameter sigmoid model, $G = a/1 + e[−(x − T_{50})/b]$, was fit to the emergence data. In the equation, G represents the cumulative emergence percentage at time x, a is the maximum emergence percentage, T_{50} is the time (in days) required for 50% of maximum emergence, and b is the slope.
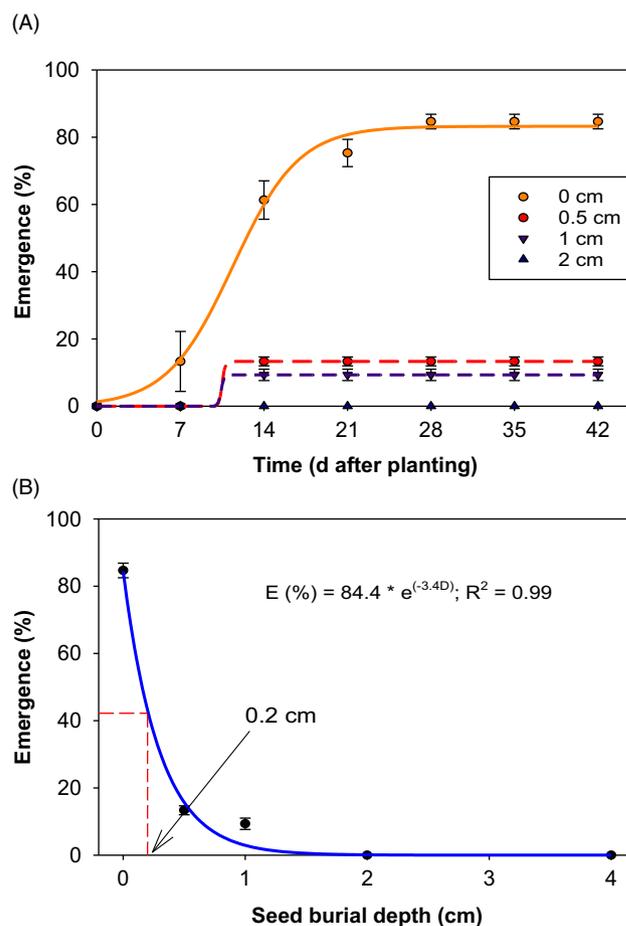

Figure 4. Effect of seed burial depth and time after planting on emergence of *Dinebra retroflexa* (A) and effect of depth on cumulative emergence (B). A three-parameter sigmoid model, $G = a/1 + e[−(x − T_{50})/b]$, was fit to the germination data. In the equation, G represents the cumulative germination percentage at time x, a is the maximum germination percentage, T_{50} is the time in days required for 50% inhibition of maximum germination, and b is the slope (B). An exponential decay model, $E = a * e^{-bD}$, was fit to the seedling emergence from different seed burial depths, where E is the emergence percentage at burial depth D, a is the maximum emergence, and b is the slope (B). The vertical bars represent the standard errors of the means.

(Chauhan et al. 2018). The greatest emergence of *D. retroflexa* under no-residue conditions indicated its potential to infest crops under clean cultivation. Results suggest that conservation agriculture with residue retention can help in managing this weed.

Table 6. Effect of wheat crop residue load on emergence of *Dinebra retroflexa*.[a]

Residue amount	Parameter estimates (±SE)			R^2
	a	*b*	T_{50}	
kg ha^{-1}	%		d	
0	82.3 (2.8)	2.8 (0.8)	6.9 (0.7)	0.98
1,000	71.8 (2.6)	3.6 (0.8)	8.4 (0.9)	0.98
2,000	61.6 (0.6)	3.0 (0.2)	10.3 (0.2)	0.99
4,000	28.3 (0.9)	2.4 (0.6)	12.8 (0.6)	0.99
8,000	2% germination (curve did not fit)			

[a]A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the emergence data. In the equation, G represents the cumulative emergence percentage at time x, a is the maximum emergence percentage, T_{50} is the time (in days) required for 50% of maximum emergence, and b is the slope.
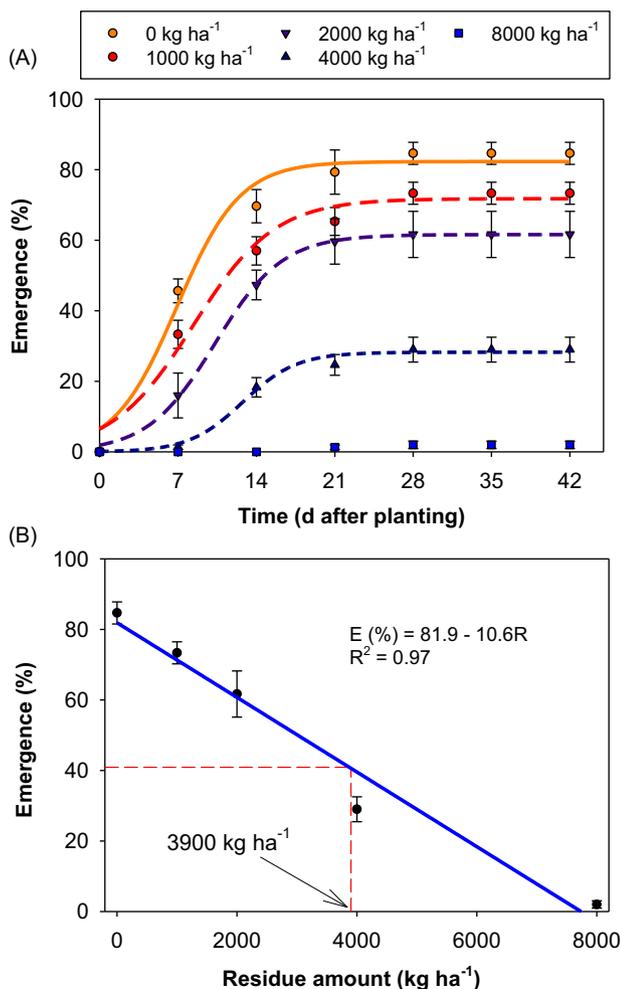

Figure 5. Effect of wheat crop residue load and time after planting on emergence of *Dinebra retroflexa* (A) and effect of residue load on cumulative emergence of *D. retroflexa* (B). A three-parameter sigmoid model, $G = a/1 + e[-(x - T_{50})/b]$, was fit to the emergence data. In the equation, G represents the cumulative germination percentage at time x, a is the maximum germination percentage, T_{50} is the time in days required for 50% inhibition of maximum germination, and b is the slope (A). A linear model ($E = a - bR$) was fit to the emergence obtained at different residue loads, where E is the emergence percentage at crop residue amount R, a is the maximum emergence, and b is the slope. The vertical bars represent the standard errors of the means.

Further, in the case of low residue–producing crops or easily degradable residue crops, planting a cover crop before the main crop to create a residue cover could be an option to suppress *D. retroflexa*.

Table 7. Effect of preemergence herbicides on seedling survival, dry matter accumulation, and percent control based on biomass of *Dinebra retroflexa*.

Herbicide treatments	Survival	Biomass	Control
	%	g pot^{-1}	%
Control	86.7	0.5205	0
Dimethanamid-P	1.7	0.0015	99
Diuron	0	0	100
Imazethapyr	5.0	0.0215	96
Isoxaflutole	0	0	100
Pendimethalin	0	0	100
Pyroxasulfone	0	0	100
S-metolachlor	0	0	100
S-metolachlor + prosulfocarb	0.8	0.003	99
Terbuthylazine	0	0	100
Triallate	0	0	100
Trifluralin	22.5	0.031	94
LSD (5%)	3.56	0.035	—

Experiment 7. Effect of Pre- and Postemergence Herbicides on Survival and Biomass

All the preemergence herbicides tested in this study provided effective control (>90%) of *D. retroflexa.* Applications of diuron, isoxaflutole, pendimethalin, pyroxasulfone, *S*-metolachlor, terbuthylazine, and triallate provided complete control (100%) of *D. retroflexa*. The application of trifluralin resulted in the maximum (23%) emergence of seedlings among all preemergence herbicides, followed by imazethapyr (5%) and dimethenamid-P (2%), indicating relatively poor control of *D. retroflexa* (Table 7). Similarly, biomass data indicated that all the tested preemergence herbicides resulted in 94% to 100% biomass reductions as compared with the control.

These results are in line with a previous Indian study, in which a 70% reduction in *D. retroflexa* density was recorded with a preemergence application of pendimethalin (750 g ha^{-1}) alone or in combination with imazethapyr (50 g ha^{-1}) in a sesame (*Sesamum orientale* L.) crop (Singh et al. 2018). Another study conducted in Queensland, Australia, found that preemergence applications of pendimethalin (1,496 g ha^{-1}) and *S*-metolachlor (960 g ha^{-1}) resulted in complete control (100%) of *D. retroflexa* in glyphosate-tolerant cotton (Iqbal et al. 2019). Patel et al. (2019) also found maximum biomass reduction (75%) of *D. retroflexa* in soybean [*Glycine max* (L.) Merr.] with the application of imazethapyr + propaquizafop (75.0 + 62.5 g ha^{-1}). Results from the present study showed the importance of preemergence herbicides for effective control of *D. retroflexa*.

All the postemergence herbicides applied at the 5- to 6- and 11- to 12-leaf stages resulted in complete control (100%) of *D. retroflexa* (data not shown). Biomass accumulated in the control treatment was 5.15 ± 0.93 g pot^{-1} at the 5- to 6-leaf stage and 8.23 ± 0.52 g pot^{-1} at the 11- to 12-leaf stage of the plant. Similar to our results, Iqbal et al. (2019) also reported that a postemergence application of glyphosate + pendimethalin (1,034 + 1,496 g ha^{-1}) resulted in 100% biomass reduction of *D. retroflexa* in glyphosate-tolerant cotton. These results indicate that *D. retroflexa* can be effectively managed with the application of commonly used postemergence herbicides. These results are based on a small number of pot-grown plants derived from a single population. Therefore, future research needs to be done to study the effectiveness of these pre- and postemergence herbicides under field conditions, as plants may be under stress and exhibiting variable sizes under field conditions, which may reduce herbicide

efficacy. Further, there is a need to rotate herbicides with different sites of action to delay the evolution of resistance in *D. retroflexa*.

In summary, seeds of *D. retroflexa* are likely to germinate over a wide range of environmental conditions. Seed germination over a wide temperature range demonstrates its potential to expand throughout Australia's summer, autumn, spring, and mild winter seasons. Poor germination in dark conditions suggests the inability of this weed species to emerge from greater depths or under a dense canopy. High germination under high soil moisture and salinity conditions indicates its potential to spread in irrigated areas and salt-affected soils of Australia. No-till cropping systems or shallow cultivation would favor its infestation. Results from this study showed that even a shallow-tillage operation may bury the seeds of *D. retroflexa* and restrict its emergence. Further, the retention of wheat residue on the soil surface creates dark conditions and acts as a barrier to its emergence; therefore, it will help in reducing its seedling emergence. Although *D. retroflexa* can be easily controlled with the application of commonly used pre- or postemergence herbicides, the chemical option should be used in addition to other management tools to avoid herbicide resistance issues in this weed species.

References

Abrol I, Yadav JSP, Massoud F (1988) Salt-Affected Soils and Their Management. Food and Agriculture Organisation (FAO) Soils Bulletin 39. Rome: FAO. 131 p

Altop EK, Mennan H, Phillippo CJ, Zandstra BH (2015) Effect of burial depth and environmental factors on the seasonal germination of bearded sprangle-top (*Leptochloa fusca* [L.] Kunth ssp. *fascicularis* [Lam.] N. Snow). Weed Biol Manag 15:147–158

Anonymous (2022) Weed Identification and Knowledge in the Tropical and Mediterranean Areas. https://portal.wiktrop.org/species/show/477. Accessed: April 20, 2022

Babiker AGT, Ahmed MK (1986) Chemical weed control in transplanted onion (*Allium cepa* L.) in the Sudan Gezira. Weed Res 26:133–137

Baskin CC, Baskin JM (1998) Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego, CA: Academic Press. 666 p

Baskin CC, Baskin JM, Chester EW (1999) Seed germination ecology of the annual grass *Leptochloa panicea* ssp. *mucronata* and a comparison with *L. panicoides* and *L. fusca*. Oecologica 20:571–577

Benvenuti S, Dinelli G, Bonetti A (2003) Germination ecology of *Leptochloa chinensis*: a new weed in the Italian rice agro-environment. Weed Res 44:87–96

Bewley JD, Bradford KJ, Hilhorst HW, Nonogaki H (2013) Germination. Pages 133–181 in Bewley JD, Bradford KJ, Hilhorst HW, Nonogaki H, eds. Seeds. New York: Springer

Bot AJ, Nachtergaele FO, Young A (2000) Land Resource Potential and Constraints at Regional and Country Levels. Rome: Food and Agriculture Organization of the United Nations, Land and Water Development Division. 114 p

[CABI] Centre for Agriculture and Bioscience International (2022) Invasive Species Compendium: Dinebra retroflexa. Wallingford, UK: CABI. https://cabi.org/isc/datasheet/113909. Accessed: April 20, 2022

Chauhan BS (2013) Seed germination ecology of feather lovegrass [*Eragrostis tenella* (L.) Beauv. Ex Roemer & JA Schultes]. PLoS ONE 8:e79398

Chauhan BS, Gill G, Preston C (2006) Influence of tillage systems on vertical distribution, seedling recruitment and persistence of rigid ryegrass (*Lolium rigidum*) seed bank. Weed Sci 54:669–676

Chauhan BS, Johnson DE (2008) Germination ecology of Chinese sprangletop (*Leptochloa chinensis*) in the Philippines. Weed Sci 56:820–825

Chauhan BS, Johnson DE (2010) The role of seed ecology in improving weed management strategies in the tropics. Adv Agron 105:221–262

Chauhan BS, Manalil S, Florentine S, Jha P (2018) Germination ecology of *Chloris truncata* and its implication for weed management. PLoS ONE 13: e0199949

Cousens RD, Baweja R, Vaths J, Schofield M (1993) Comparative biology of cruciferous weeds: a preliminary study. Pages 376–380 in Proceedings of the 10th Australian and 14th Asian-Pacific Weed Conference. Brisbane, Australia: Weed Society of Queensland

Farooq M, Imran A, Alam S, Naseem S, Riaz S, Shaukat SF (2015) Friction and wear assessment of yttria stabilised zirconia thermal barrier coatings produced by plasma spraying method. J Fac Engin Technol 22:1–7

Fernando N, Humphries T, Florentine SK, Chauhan BS (2016) Factors affecting seed germination of feather fingergrass (*Chloris virgata*). Weed Sci 64: 605–612

Genstat (2021) Genstat for Windows. 21st ed. Hemel Hempstead, UK: VSN International

Ghobrial GI (1981) Weed control in irrigated dry seeded rice. Weed Res 21: 201–204

Hamada AA, Koch W, Hamdoun A, Kunisch M, Sauerborn J (1993) Effect of temperature, light, and simulated drought on the germination of some weed species from Sudan. Angew Bot 67:52–55

Ibrahim AAS (1984) Weed competition and control in sugarcane. Weed Res 24:227–231

Iqbal N, Manalil S, Chauhan BS, Adkins SW (2019) Investigation of alternate herbicides for effective weed management in glyphosate-tolerant cotton. Arch Agron Soil Sci 65:1885–1899

Karar RO, Mohamed BF, Marrs RH (2005) Factors influencing the weed flora in the Gezira Scheme, Sudan. Weed Res 45:121–129

Llewellyn RS, Ronning D, Ouzman J, Walker S, Mayfield A, Clarke M (2016) Impact of Weeds on Australian Grain Production: The Cost of Weeds to Australian Grain Growers and the Adoption of Weed Management and Tillage Practices. Report for GRDC. Barton, Australia: CSIRO. 112 p

Mahajan G, Chauhan BS (2020) Weed biology—a required foundation for effective weed management. Weeds 2:30–34

Mahajan G, Matloob A, Walsh M, Chauhan BS (2018) Germination ecology of two Australian populations of African turnip weed (*Sisymbrium thellungii*). Weed Sci 66:752–757

Mahajan G, Prasad A, Chauhan BS (2021) Seed germination ecology of Sumatran fleabane (*Conyza sumatrensis*) in relations to various environmental parameters. Weed Sci 69:687–694

Manalil S, Werth J, Jackson R, Chauhan BS, Preston C (2017) An assessment of weed flora 14 years after the introduction of glyphosate-tolerant cotton in Australia. Crop Past Sci 68:773–780

Michel BE, Radcliffe D (1995) A computer program relating solute potential to solution composition for five solutes. Agron J 87:126–130

Munirathnam P, Kumar KA (2014) Survey of weed flora in major crops of Nandyal region in Kurnool district of Andhra Pradesh. J Res ANGRAU 42:142–146

Nayak SK, Satapathy KB (2015) Diversity, uses and origin of invasive alien plants in Dhenkanal district of Odisha, India. Int Res J Biol Sci 4:21–27

Patel A, Spare N, Malgaya G (2019) Bio-efficacy of post emergence herbicides against weed control in soybean. Int J Curr Microbiol 8:1964–1974

Rengasamy P (2002) Transient salinity and subsoil constraints to dryland farming in Australian sodic soils: an overview. Aust J Exp Agric 42: 351–361

Roundy BA, Biedenbender SH (1996) Germination of warm-season grasses under constant and dynamic pressures. J. Range Manage 49: 425–431

Satyamurty TVC (1985) Life history of *Dinebra retroflexa*. Acta Bot Ind 13: 266–268

Singh R, Ghosh D, Dubey RP, Singh VP (2018) Weed control in sesame with pre-emergence herbicides. Ind J Weed Sci 50:91–93

Singh S, Mahajan G, Singh R, Chauhan BS (2021) Germination ecology of four African mustard (*Brassica tournefortii* Gouan.) populations in the eastern region of Australia. Weed Sci 69:461–467

Snow N, Peterson PM (2012) Nomenclature notes on *Dinebra*, *Diplachne*, *Disakisperma*, and *Leptochloa* (Poaceae: Chloridoideae). Phytoneuron 71:1–2

Tanji A (2020) Notes about two summer annual grass weeds in Morocco: *Dinebra retroflexa* and *Cenchrus longispinus* (Poaceae). Fl Medit 30: 113–119

Welbaum GE, Tissaoui T, Bradford KJ (1990) Water relations of seed development and germination in muskmelon (*Cucumis melo* L.) III. Sensitivity of germination to water potential and abscisic acid during development. Plant Physiol 92:1029–1037

Weller SL, Florentine SK, Chauhan BS (2019) Influence of selected environmental factors on seed germination and seedling emergence of *Dinebra panicea* var. *brachiata* (Steud.). Crop Prot 117:121–127