ABSTRACT - The species *Euphorbia dracunculoides* and *Astragalus* are problematic weeds of arid chickpea in the chickpea monocropping system in Pakistan. The influence of various ecological factors on germination and seedling emergence characteristics of these weeds was determined under laboratory conditions. The results suggested that seed germination of both species was 50% at 15 °C under light conditions, and germination decreased when the temperature was increased. The increase in drought stress from 2.5 to 15% significantly decreased germination of *E. dracunculoides* and *Astragalus* spp. Both species failed to germinate at the osmotic potential of -3.02 MPa. The increase in field capacity from 25 to 100% increased emergence percentage and emergence index of both weeds. A pH range of 6 to 9 did not influence seed germination of both species and they were able to germinate at a wide range of pH conditions. Both weeds were very sensitive to salinity; however, a few seeds (10%) of *Astragalus* spp. germinated even at a 150 mM sodium chloride concentration. To check the effect of burial depth, seeds were placed in pots under seeding depths of 0 to 6 cm at an interval of 1 cm, respectively. Maximum emergence was attained at the soil surface and emergence declined with increasing depths. Seedling emergence of *E. dracunculoides* was higher than that of *Astragalus* spp. at all burial depths. Studies on germination ecology of these two weeds will offer insights into their behavior under different environmental conditions. Their germination responses and growth patterns under different ecological factors will help us to design an efficient management strategy to control these two troublesome weeds.

**Keywords:** temperature, burial depth, light, management, emergence, salinity.

RESUMO - As espécies *Euphorbia dracunculoides* e *Astragalus* são plantas daninhas problemáticas do grão-de-bico no sistema monocultivo desta cultura no Paquistão. Em condições de laboratório, determinou-se a influência de vários fatores ecológicos nas características de germinação e emergência de plântulas dessas plantas daninhas. Os resultados sugeriram que a germinação de sementes de ambas as espécies foi de 50% a 15 °C sob condições de luz, e a germinação diminuiu com o aumento da temperatura. O aumento do estresse hídrico de 2,5 para 15% causou a diminuição significativa da germinação de *E. dracunculoides* e *Astragalus* spp. Ambas as espécies não germinaram no potencial osmótico de -3,02 MPa. O aumento na capacidade de campo de 25 para 100% aumentou a porcentagem de emergência e o índice de emergência de ambas as plantas daninhas. A variação do pH de 6 a 9 não influenciou a germinação de sementes de ambas as espécies, que foram capazes de germinar em uma ampla gama de condições de pH.
Ambas as plantas daninhas foram muito sensíveis à salinidade; no entanto, algumas sementes (10%) de Astragalus spp. germinaram mesmo em uma concentração de cloreto de sódio de 150 mM. Para verificar o efeito da profundidade de semeadura, as sementes foram colocadas em vasos com profundidades de semeadura de 0 a 6 cm, em um intervalo de 1 cm, respectivamente. Foi obtida máxima emergência na superfície do solo e houve diminuição da emergência com o aumento da profundidade. A emergência de plântulas de E. dracunculoides foi maior que a de Astragalus spp. em todas as profundidades de semeadura. Estudos sobre a ecologia da germinação dessas duas plantas daninhas poderão elucidar seu comportamento sob diferentes condições ambientais. As respostas de germinação e os padrões de crescimento dessas espécies sob diferentes fatores ecológicos serão úteis para a elaboração de uma estratégia de gerenciamento eficiente para controlar essas duas plantas daninhas problemáticas.

Palavras-chave: temperatura, profundidade de semeadura, luz, manejo, emergência, salinidade.

INTRODUCTION

Euphorbia dracunculoides Lamk. is an annual, medium sized herb with a smooth stem. It belongs to the family Euphorbiaceae. It is usually 15-40 cm tall and its stem is often much branched from the base (Wang et al., 2014). This species has been reported in cultivated and sandy fields of Pakistan, Egypt, Iraq, Kuwait, Saudi Arabia, Afghanistan, India, and tropical Africa (Shanee et al., 2011). Euphorbia dracunculoides density of 40 plants m-2 can cause chickpea grain yield losses by up to 63% (Tanveer et al., 2015). Astragalus spp. is an annual, xerophytic shrub, which belongs to the family Fabaceae, and it normally achieves up to 40 cm height (Drobná, 2010). Genus Astragalus is found in North and South America, Asia, Europe, northern Africa, and Australia (Naghiloo et al., 2012). Both species are the most troublesome weeds of rainfed chickpea (Ikram et al., 2014). The salient features of this area include moisture shortage as a result of inadequate and erratic rains, low-temperature stress (frost) during December-January, marginal land, no use of fertilizers, and presence of weeds (Sijapati and Bhatt, 2012).

Understanding the biology and ecology of a weed is needed for effective management (Koger et al., 2004; Fenner and Thompson, 2005; Chauhan, 2013). Knowledge based on research of critical life stage and seed germination offers a comprehensive explanation of plant development, seed dormancy patterns, ecological adaptation traits, distribution, and management strategies (Fandrich and Mallory-Smith, 2006). Various studies on germination ecology stated that many factors, such as salinity, temperature, seed age, sowing depth, pH, light, and moisture, influence germination and emergence of a weed species (Chauhan and Johnson, 2008b, 2009; Nakamura and Hossain, 2009).

Optimum temperature determines the ecological limitation and adaptation for the geographical distribution of a species (Nerson, 2007; Turkoglu et al., 2009). Light is one of the environmental factors that can influence dormancy. Seeds of some species require light for germination and light exposure of less than a minute and, for some species, less than a second is enough to induce germination (Milberg et al., 1996). pH is also an important environmental factor that can severely restrict germination as a result of its effects on enzyme activity (Ali et al., 2013). In this context, salts create water deficit along nutritional imbalance and are another reason for an unfavorable environment for seed germination (Achuo et al., 2006). Water stress arising from drought is probably the most noteworthy abiotic feature limiting germination (van der Berg and Zeng, 2006). In case of severe drought, germination stops. As a result, the embryo of seeds cannot develop into radicle and plumule. Hence, moisture availability has a very dynamic role in seed germination (Hillel, 1972). Almansouri et al. (2001) reported that moisture and temperature are critical factors for seed germination. Seeding depth is one of the most important environmental factors affecting seed germination and seedling emergence because seeds are present in soil at different depths (Liu et al., 2011). The behavior of seed germination changes with an increase or decrease in soil depth environments. The ideal soil depth varies with species (Gulshan and Dasti, 2006; Ali et al., 2013).

Euphorbia dracunculoides has been previously studied for its phytotoxicity (Shanee et al., 2011). However, those studies did not address the germination ecology of E. dracunculoides. On the other hand, there is no work reported on Astragalus spp. Therefore, a significant understanding...
through ecological aspects is necessary. These weeds were particularly chosen because these are ubiquitous in the typical rainfed zone of Pakistan and known to occur in the mono cropping system for chickpea. Given the aforesaid importance of *Euphorbia dracunculoides* and *Astragalus* spp., the objective of present project was to understand the germination response of *E. dracunculoides* and *Astragalus* spp. to various environmental factors.

**MATERIALS AND METHODS**

**Collection of weed seeds**

Seeds of both weeds (*E. dracunculoides* and *Astragalus* spp.) were collected from plants at maturity from District Khushab of Punjab province, Pakistan, in October 2010. The collected seeds were isolated from fruits of *E. dracunculoides* and pods of *Astragalus* spp. and undesirable materials and immature/damaged seeds in the laboratory. These healthy, mature and uniform seeds were stored in paper bags under normal laboratory conditions after drying.

**General germination test protocol**

For all laboratory experiments, seeds were rinsed thoroughly with sterilized water four times and placed on double layered Whatman No. 10 filter papers in sterilized Petri dishes of 9 cm diameter. Initially, 5 mL of distilled water/respective solution of each treatment was applied per Petri dish and after this, the solution was added whenever needed. Each experiment was laid out in a completely randomized design with four replications, and 25 seeds were allocated per Petri dish.

**Temperature**

To evaluate the influence of temperature, 25 seeds for each species were evenly placed on double layer of filter papers in Petri plates, which were then placed in a germinator at the temperatures of 10, 15, 20, and 25 °C.

**Light**

The seeds of both species were evenly placed on Whatman no 10 filter papers in Petri dish and kept uncovered for complete light exposure. Similarly, to evaluate the effect of darkness, Petri dishes with seeds on double layered filter papers were wrapped with a single layer of aluminum foil and then placed in a germinator at 15 °C.

**pH**

The effect of pH on germination of *E. dracunculoides* and *Astragalus* spp. was studied by using buffer solutions of pH 6.0, 7.0, 8.0 and 9.0, prepared as described by Reddy and Singh (1992). These pH ranges were selected to reflect the prevailing soil pH conditions in southern Punjab, Pakistan. A 2 mM solution of MES [2-(Nmorpholino) ethanesulfonic acid], HEPES [N-(2-hydroxymethyl) piperazine-N-(2-ethanesulfonic acid)], and tricine [N-tris(hydroxymethyl) methylglycine] were adjusted to pH 6, 7-8, and 9 with 1 N NaOH, respectively. Deionized water was used as a control treatment. Petri dishes were placed in a germinator at 15 °C.

**Salt stress**

To assess the germination ability of *E. dracunculoides* and *Astragalus* spp. under different levels of salt stress, 25 seeds of each species were evenly placed on Whatman No. 10 filter papers in 9 cm diameter Petri dish. Sodium chloride solutions of 0 (control), 25, 50, 75, 100, 125, and 150 mM concentrations were added to each Petri dish separately. These ranges of NaCl were selected to reflect the level of salinity occurring in the soils of southern Punjab. The Petri dishes were sealed with a strip of parafilm to prevent moisture loss and placed in a germinator at 15 °C.
Drought stress

The germination response of *E. dracunculoides* and *Astragalus* spp. under eight levels of drought stress was also assessed under laboratory environments. PEG-8000 was used as a drought stimulator with seven water stress levels. These were the osmotic stress levels applied: 0% (control), 2.5% (-0.17 MPa), 5.0% (-0.47 MPa), 7.5% (-0.91 MPa), 10.0% (-1.48 MPa), 12.5% (-2.18 MPa), and 15.0% (-3.02 MPa). These concentrations were developed according to the equation proposed by Michel (1983). After the application of the required treatments, Petri dishes were kept in a germinator at a temperature of 15°C.

Field capacity

In this experiment, four field capacity levels of 25%, 50%, 75%, and 100% were maintained while using soil as the sowing medium. Soil was collected from the area where there were no weeds previously. The soil was then dried properly and placed into 14 cm diameter plastic pots. The analysis of soil showed that it was sandy loam soil with 0.7% carbon and pH of 7.1. Twenty-five seeds of each weed were evenly placed in plastic pots and different levels of field capacity were maintained (Tanveer et al., 2013). The pots were placed in a greenhouse. The average minimum and maximum temperatures in the greenhouse during the experiment were 15°C and 20°C. Field capacity was determined as per the procedure followed by Tanveer et al. (2013) by using the following formula:

\[
\text{Field capacity} = \frac{\text{Saturation percentage}}{2}
\]

The plastic pots were filled with soil and then water was applied. After being watered, the pots were weighed. Weight was measured of the pots containing moisture contents equal to 100%, 75%, 50%, and 25% field capacities (Tanveer et al., 2013).

Seeding depth

Twenty-five seeds each of *E. dracunculoides* and *Astragalus* spp. were separately placed in 14 cm diameter plastic pots on the soil surface (0 cm) or covered to depths of 1, 2, 3, 4, 5, and 6 cm. A mixture of sand, silt, and clay was used as media for germination in the pots. Initially, 100 mL distilled water was given to each pot with an irrigation shower so as to avoid soil disturbance. The average minimum and maximum temperatures recorded in the greenhouse during the experiment were 15°C and 20°C. Daily germination counts were performed for 3 weeks. Non-germinated seeds were subjected to the tetrazolium salt test to check viability. In the seed burial depth experiment, seedling emergence was considered as such when a cotyledon became visible on the surface. We recorded seedling emergence data up to 30 days.

The germination/emergence index (GI/EI) was assessed as formula given by the Association of Official Seed Analysts (AOSA, 1990):

\[
GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}
\]

Germination energy (GE) was determined as suggested by the Association of Official Seed Analysts (AOSA, 1990):

\[
GE = \frac{\text{Total germinated seeds at 4th day}}{\text{Total seeds}} \times 100
\]

All experiments were repeated within 20 days after the end of the first run. The data from the repeated experiments were analyzed by using the ANOVA function of the MSTAT statistical computer package and least significance difference (LSD) at 5% probability was used to compare the treatment means (Steel et al., 1997).
RESULTS AND DISCUSSION

Effect of temperature

Germination of *E. dracunculoides* and *Astragalus* species was significantly (P<0.05) affected by the temperatures tested (Figure 1) and it was significantly higher at 15 °C (50% for *E. dracunculoides* and 40% for *Astragalus* species) than at 10, 20, and 25 °C. GE and GI of both weeds were maximum at 15 °C and then decreased with a further increase or decrease in temperatures (Table 1). Similarly to the species *E. dracunculoides* and *Astragalus*, other weed species, including *Caragana microphylla* (Zhu et al. 2004) and *Rhynchosia capitata* (Ali et al. 2013), germinated over a wide range of temperature conditions.

![Figure 1 - Effect of temperature on seed germination of Euphorbia dracunculoides (A) and Astragalus spp. (B).](image)

The ability of both weeds to germinate over the tested temperature range suggests that the weeds could emerge throughout the growing season of rainfed chickpea. Such a broad adaptation of these weeds to temperature also provides opportunities for seed production and weed proliferation.
Table 1 - Effect of temperature on germination traits of Euphorbia dracunculoides and Astragalus spp.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Germination energy E. dracunculoides</th>
<th>Germination index E. dracunculoides</th>
<th>Germination energy Astragalus spp.</th>
<th>Germination index Astragalus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.00 c</td>
<td>10.00 b</td>
<td>0.08 d</td>
<td>1.25 c</td>
</tr>
<tr>
<td>15</td>
<td>13.00 a</td>
<td>18.00 a</td>
<td>2.12 a</td>
<td>2.44 a</td>
</tr>
<tr>
<td>20</td>
<td>9.00 b</td>
<td>16.00 a</td>
<td>1.65 b</td>
<td>1.93 b</td>
</tr>
<tr>
<td>25</td>
<td>3.00 c</td>
<td>4.00 c</td>
<td>0.83 c</td>
<td>0.73 d</td>
</tr>
</tbody>
</table>

Mean values with a matching letter in a column did not fluctuate significantly as per the LSD test (p<0.05).

**Effect of light and darkness**

Seed germination of E. dracunculoides and Astragalus species was significantly affected by light conditions. Germination (Figure 2) values for the weed species were 66% for E. dracunculoides and 44% for Astragalus species; GE and GI (Table 2) were significantly greater in light/dark (10/14 hr) than in the dark. Reports on germination response to light are inconsistent. For example, the higher germination of these weed seeds under light indicates that emergence will be favored by the seeds that are present at or near the soil surface, i.e., the condition that occurs under no-till chickpea cropping systems. Similarly to the results in the present study, seed germination of Sonchus oleraceus (Chauhan et al. 2006b) and Faloua villosa (Gina and Joseph, 2003) was stimulated by light instead of darkness. Our results are further supported by the findings of Gracia et al. (2005), who reported that absence of light inhibited germination of Triglochin maritime Chauhan et al. (2006c) reported that in Rapistrum rugosum, germination was 87% under light/dark and 76% in the dark. They concluded that seed germination was stimulated by light. Lu et al. (2006) found that Crofton weed (Eupatorium adenophorum) was moderately photoblastic, with 17% germination occurring in the dark. It suggests that seed germination to light could be variable within weed species.

In the present study, seed germination of E. dracunculoides and Astragalus species was intensely stimulated by light, which means that seeds of these species are positively photoblastic. When seeds of both weeds were exposed to 10 h photoperiod, they showed significantly higher GE and GI compared to those seeds that remained under complete darkness. Greater seed germination of the species E. dracunculoides and Astragalus under light conditions suggests that germination and emergence of both weeds in the field will be ideal at or near the soil surface. No-till systems, in which a large proportion of weed seeds will be near the soil surface, would favor seed germination/emergence and their subsequent management.

**Effect of pH**

Seed germination of E. dracunculoides and Astragalus species was significantly affected by the tested range of pH solutions, and it varied from 52 to 25% in E. dracunculoides and 38 to 16% in Astragalus over the pH range of 6 to 9 (Figure 3). Germination of both weeds at pH 6 to 9 indicates that pH may not be a limiting factor for germination of these weeds. This kind of character leads a plant to become an invasive weed (Watanabe et al., 2002). Germination of Galium tricornutum (Dandy) was found over a pH range from 4 to 10 (Chauhan et al., 2006a) and Solanum nigrum at neutral pH showed a high percentage of germination (Suthar et al., 2009). A higher germination percentage of E. dracunculoides than that of Astragalus over a pH range of 6 to 9 explains its wide range of adaptability. The results show that both species are slightly sensitive to pH 9 while both germinated well at other pH levels (Table 3). This kind of behavior of weeds suggests that they can adapt to a wide range of soil conditions.

**Effect of salt stress**

Germination of the species E. dracunculoides and Astragalus decreased after an increase in NaCl concentrations from 0 to 150 mM although germination of the former weed was completely inhibited at 125 and 150 mM NaCl (Figure 4). Seed germination of Astragalus spp. was more
tolerant to high concentrations of NaCl than that of *E. dracunculoides*, which suggests that, under saline conditions, more proportions of *Astragalus* seeds may germinate, which could be a key feature of this species that enables it to colonize in saline areas more rapidly than *E. dracunculoides*. There was a linear decrease (Table 4) in GE and GI after an increase in salinity from 0 to 150 mM in *E. dracunculoides* and from 25 to 150 mM in *Astragalus*. These results are line with those of Chauhan and Johnson (2008a), who reported that *Corchorus olitorius* and *Melochia concatenata* were moderately tolerant to salt stress.

**Effect of drought stress**

In both weeds, seed germination declined with decreasing osmotic potential, and seeds of neither species germinated at the osmotic potential of -3.02 MPa (Figure 5). Data also suggest
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**Figure 4** - Effect of salt stress on seed germination of *Euphorbia dracunculoides* (A) and *Astragalus* spp. (B).

**Table 4** - Effect of salt stress on germination traits of *Euphorbia dracunculoides* and *Astragalus* spp.

<table>
<thead>
<tr>
<th>Salt stress (mM)</th>
<th>Germination energy</th>
<th>Germination index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. dracunculoides</em></td>
<td><em>Astragalus</em> spp.</td>
</tr>
<tr>
<td>0</td>
<td>22.00 a</td>
<td>15.00 a</td>
</tr>
<tr>
<td>25</td>
<td>8.00 b</td>
<td>15.00 a</td>
</tr>
<tr>
<td>50</td>
<td>6.00 b</td>
<td>5.00 b</td>
</tr>
<tr>
<td>75</td>
<td>0.00 c</td>
<td>3.00 bc</td>
</tr>
<tr>
<td>100</td>
<td>0.00 c</td>
<td>2.00 bc</td>
</tr>
<tr>
<td>125</td>
<td>NG</td>
<td>2.00 bc</td>
</tr>
<tr>
<td>150</td>
<td>NG</td>
<td>1.00 c</td>
</tr>
</tbody>
</table>

Mean values with a matching letter in a column did not fluctuate significantly as per the LSD test (p<0.05). NG: Not germinated.

**Effect of field capacity**

The progressive decrease in field capacity declined the emergence percentage of the species *E. dracunculoides* and *Astragalus* (Figure 6). At the 100% field capacity level, emergence was 60%
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weeds such as *E. adenophoum* and taxasweed *Caperonia palustris* are sensitive to drought stress (Koger et al., 2004; Lu et al., 2006); by contrast, other weed species such as *Hibiscus tridactylites* and *Solanum sarrachoides* were tolerant to drought stress (Zhou et al., 2005; Chauhan, 2016).

**Effect of burial depth**

Seed burial depth had a significant influence on the seedling emergence of *E. dracunculoides* and *Astragalus* (Figure 7). As the burial depth increased, seedling emergence decreased, and no

**Table 6 - Effect of field capacity on emergence traits of Euphorbia dracunculoides and Astragalus spp.**

<table>
<thead>
<tr>
<th>Field capacity (%)</th>
<th>Emergence energy</th>
<th>Emergence index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. dracunculoides</em></td>
<td><em>Astragalus spp.</em></td>
</tr>
<tr>
<td>25</td>
<td>0.00 b</td>
<td>0.00</td>
</tr>
<tr>
<td>50</td>
<td>0.00 b</td>
<td>0.00</td>
</tr>
<tr>
<td>75</td>
<td>0.00 b</td>
<td>0.00</td>
</tr>
<tr>
<td>100</td>
<td>5.00 a</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Mean values with a matching letter in a column did not fluctuate significantly as per the LSD test (p<0.05).
Astragalus seedlings emerged when the seeds were buried at a depth of 5 and 6 cm. Emergence was higher (78% for *E. dracunculoides* and 38% for *Astragalus*) for seeds placed on the soil surface followed by seeds buried at 1 cm. There was a higher germination/emergence rate (78%) of *E. dracunculoides* in the pot trial as compared to that found in Petri dishes in the laboratory. It may have been due to the release of leachates in the presence of soil (Houseman and Mahoney, 2015), which may have increased the germination of *E. dracunculoides*. Maximum GE and GI values were recorded for both weed species when seeds were placed on the soil surface (Figure 7). There are several pieces of evidence of maximum seed germination at depths of 0 to 2 cm (Chauhan and Johnson, 2008b; Xiao et al., 2010) with the lowest germination at 6 cm (Liu and Han, 2008). Decreased seedling emergence resulting from increased burial depth has been reported in *Asphodelus tenuifolius*, an important weed of arid areas (Tanveer et al., 2014). The higher rate of seedling emergence of *E. dracunculoides* as compared to *Astragalus* at all burial depths could have been due to large seed size, which is associated with higher tolerance to adverse conditions (Tanveer et al., 2013). On the other hand, species such as *Astragalus* (Figure 8) may not have sufficient energy reserves to support hypocotyls elongation. Greater seedling emergence from seeds placed on the soil surface suggests that no-till farming systems which are being used in the rainfed chickpea mono-cropping system of Pakistan would favor the emergence of *E. dracunculoides* and *Astragalus*.

![Figure 7 - Effect of seeding depth on seed emergence of Euphorbia dracunculoides.](image)

![Figure 8 - Effect of seeding depth on emergence of Astragalus spp.](image)
Based on the results of the present study, it can be concluded that *E. dracunculoides* and *Astragalus* ssp. have the ability to germinate over a broad range of environmental conditions. Their tolerance against environmental stresses make these weed species troublesome and difficult to control. As these species are sensitive to dark conditions, and germination decreases by increasing depth, deep tillage will lead towards deep placement of these weed seeds, hence making them dormant or minimizing their germination. Thus, by observing their growth patterns, an environmentally friendly management strategy should be designed to focus on cultural controls.

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