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ENVIRONMENTAL FACTORS INFLUENCING *LOLIUM TEMULENTUM* L. (DARNEL RYEGRASS) SEED GERMINATION

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Abstract – Darnel ryegrass (*Lolium temulentum* L.) is an annual long-day plant belonging to the Poaceae family, that is common in grain fields worldwide. *L. temulentum* is a valuable model grass species for studying stress in forage and turf grasses. This study was conducted to assess the impact of critical environmental factors (temperature, light, pH, and salinity) on the seed biology of *L. temulentum*. The findings of this study indicated that the seeds of this weed germinate after three days at temperatures between 20 °C and 30 °C, 14 days at 10 to 15 °C, and 28 days at 5 ° C. Furthermore, at a temperature of 35 °C to 40 °C, the seeds did not germinate for 28 days. After 14 days, this species' most significant germination percentage was 91.6 % at 10 °C and 20 °C. Seeds did not germinate when incubated for 14 days under continuous darkness, whereas germinated at 32.5 % when subjected to light for 12 h daily. Also, the results showed no significant effect of examined pH (4-10) and salinity (0, 25, 50, 100, 150, 200, and 250) levels on seed germination of *L. temulentum*. The information gained from the analysis will provide a valuable biological plant germination resource that will be used to develop approaches as to how the plant can improve under abiotic stress factors.

Keywords: light, pH, salinity, temperature, weed.

Introduction

Darnel Ryegrass (*Lolium* spp.) is a bothersome weed in agricultural production globally, and it is moreover a valued cover crop, turf and cultivated forage species. High adaptive potential and diversity are identified to contribute to its achievement as a weed species and generate difficulties in precise species identification in fields (Maity et al., 2021). The genus of *Lolium* (Poaceae) comprises of several perennial and annual species. *Lolium* spp. are native to North Africa, Europe and temperate Asia, but have spread over the last 200 years to North and South America, New Zealand, Australia and southern parts of Africa. *Lolium* spp. were moved to new ares mainly as turf, pasture plants and cover crops, in contaminated livestock feed and commercial crop seed. Morphology of *Lolium* is very similar among members of the genus (Matzrafi et al., 2021).

One of the *Lolium* species *Lolium temulentum* L. is of global agricultural significance as both weeds and as pasture crops. Furthermore, it is a significant irritant in developing countries, and it is included among the 'Worst Weeds' in the world (Senda & Tominaga, 2004). The succulent weed *L. temulentum* originated in the Mediterranean and spread to temperate countries where wheat and grains are grown. Its spread into tropical regions in many countries is hindered by extended periods of elevated temperatures and little humidity (Holm et al., 1991). Due to a lack of studies, there is not much information on this plant's seed germination and emergence. Like other grass weeds of winter crops in temperate regions, it reproduces by seed. Low temperatures and high soil moisture promote its germination and growth; however,

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it can withstand extremely low temperatures (Holm et al., 1991). *L. temulentum* also failed to emerge at 10 cm seeding depth, according to Tanveer et al. (2010), and its emergence was reduced to 1 cm depth. Overgrown weeds in orchards are particularly troublesome in the first few years of a tree's existence. If the presence of herbs is not adequately handled, it can have severe repercussions since weeds can increase the insect activity and cause a risk of fire in the summer when conditions are dry (Sakit Alhaithloul, 2019).

Environmental stress factors have important effects on the growth of weeds. Temperature, one of the environmental stress factors, adversely affects plants in several ways, including plant germination, biomass, flower, and seed development. During heat stress, elements such as proteins, membranes, and mitochondria in plant cells can be damaged. With the effect of temperature stress, changes in photosynthesis, water and nutrient uptake, and changes in evapotranspiration are observed in plants. Similarly, lighting is a factor that can directly affect the photosynthetic activities and development of plants (Martin et al., 2021).

Lighting also plays a vital role in the development of weeds. The stress factor of light has a crucial role, especially in the germination phase. Although the effect of light on the germination rate varies according to the weed species, it also affects the *L. temulentum*, and the factor of light can disrupt the weed seed dormancy. Along with some studies, an attempt has been made to prevent the lighting factors that stimulate the germination of weeds by applying mulch (Botto et al., 1998; Kegode et al., 1998; Singh & Singh., 2009; Zimdahl, 2018).

Due to the lack of studies on biology and the effect of environmental conditions on seed germination, this work was conducted to study the impact of some environmental factors (temperature, light, pH, and salt stress) on the seed germination of the *L. temulentum*. With the change in the soil's pH level, there can be differences in the existing plants in the area and the species that will come to the area where the change is experienced. When there is a pH change, the competitive situation among plants can also change (Singh & Singh., 2009). So, it can be said that pH is effective in the formation of weed flora and the germination rate of weed species in a region (Alm et al., 1993; Forcella, 1993; Singh & Singh., 2009). The stress factor of salinity, which is one of the stress factors, also affects the development and survival of plants. The salinity factor affects plant growth and development as it causes a nutritional imbalance with excessive intake of ions such as sodium (Na^+) and chloride (Cl^-) (Isayenkov & Maathuis, 2019).

Our objective was to determine the effects of environmental factors (light, temperature, pH and salinity levels) on seed germination of *L. temulentum* populations. The results of the study would contribute to develop suitable and effective management strategies against the weed species.

Materials and Methods

Experimental site

This study was conducted to examine the effect of various environmental factors on *Lolium temulentum* L. seed germination. This weed's seeds were collected in the summer of 2020 from naturally ripened plants in date palm orchards in Southern Iraq; seeds were put in paper bags and stored in the lab at 20-25 °C until the experiment began. The study took place at Erciyes University Faculty of Agriculture in Plant Protection Department Herbology Laboratories in Kayseri –Türkiye from November 2020 to March 2021.

General seed germination tests

Seed preparation

L. temulentum seeds collected from Iraq agricultural lands were brought to Erciyes University laboratories in Türkiye. Those that are not healthy and damaged from the seeds were removed, they were not used in the experiment. In the experiment, seeds with a smooth appearance and no damage were preferred.

The seeds are stripped of any appendages or other items that must be removed. The seeds were cleansed once again, and any broken or rotting seeds and any pollutants overlooked in the previous phase were removed in this step. After that, the seeds were sterilized for 15 minutes in a safety cabinet with fluctuating UV light (Equipped by Berner International GmbH, Germany).

Germination of seeds

In order to examine the ecology of the seeds, *L. temulentum* seeds were subjected to ecological tests. Before testing the seeds, 30 *L. temulentum* seeds counted for each Petri dishes dish were kept separately in sodium hypochlorite solution (1%) for 1 minute and then washed 5-6 times with distilled water. Seeds were placed in Petri dishes (9 cm in diameter) containing double filter paper (Whatman No. 1), and then 5 ml of distilled water was added to the dishes. Petri dishes were covered with parafilm, since it is thought that there will be moisture loss from the Petri dishes with the temperature. Seed germination was examined at 1, 3, 7, 14, and 28 days with the criterion for germination being visible protrusion of the radicle (Göncü, 2013). Seeds were considered germinated when the radicle had emerged > 2 mm, and the radical protrusion was used to determine seed germination (Isik et. al., 2016).

Environmental conditions

Temperature

There is a certain temperature requirement during the germination period, which is the first stage of the development of plants. To determine the temperature required for the germination of *L. temulentum* seeds, different temperatures were applied to the seeds.

For 28 days, three replications of Petri dishes containing 30 sterilized seeds were placed in an incubator at varying temperatures (5, 10, 15, 20, 25, 30, 35, and 40 °C). The water level was frequently monitored to avoid drought, especially at elevated temperatures. The germination percentage was determined after 1, 3, 7, 14, and 28 days (Göncü, 2013). Germination percentages are calculated on the total of seeds put in Petri dishes at day 0. After the counts were completed, germination rate (G_{max}) and duration (T_{10}, T_{25}, T_{50} and T_{90}) values were calculated. According to this:

$$G_{max} = (G / T) \times 100$$

where G means number of total germinated seeds,
T means number of total seeds in experiment
T_{10}=Time to %10 of G-max or germinated seeds; T_{25}=Time to %25 of G-max or germinated seeds; T_{50}=Time to %50 of G-max or germinated seeds; T_{90}=Time to %90 of G-max or germinated seeds.

Light

The effect of light on seed germination were investigated under two conditions: a 12-hour daily photoperiod (light) and continuous darkness with 25/15 °C day/night temperature fluctuations (Singh & Singh, 2009). Petri dishes are coated with two layers of aluminum foil for incubation under dark conditions (Baskin & Baskin, 2014). The germination of the light treatment was monitored daily, and after 14 days of incubation, the final germination percentage for both the dark and light treatments was calculated.

pH

One of the necessary conditions for seeds to germinate in natural environments is the appropriate pH level. Seed germination, as influenced by pH, was evaluated using buffer solutions of pH from 4 to 10 prepared according to the method described by Chachalis & Reddy (2000). Pure water was used to adjust the pH. The acidity and alkalinity levels of the solution were adjusted by adding hydrogen chloride and sodium hydroxide to the pure water.

The impact of pH on seed germination was tested using buffer solutions of 4, 5, 6, 7, 8, 9, and 10 pH, which were measured by pH/mV desktop meter, Basic, equipped by mrc company, UK. After 14 days of maintaining Petri dishes with seeds at an alternating temperature of 25/15 °C, the germination percentage was determined.

Salinity

Seed germination as influenced by salt stress was evaluated using sodium chloride (NaCl) solutions of 0, 25, 50, 100, 150, and 200, and 250 mM. Seeds were placed in Petri dishes with 5-mL solutions of 0, 25, 50, 100, 150, 200, and 250 mM NaCl, which are produced by dissolving 0.375, 0.6, 1.45, 2.2, 2.925, and 3.65- g of NaCl in 250 mL of distilled water, respectively. After 14 days of incubation at different temperatures of 25/15 °C, the germination percentage is determined.

Measurement

Seed germination percentage (%): The proportion of seeds that germinate was estimated using the following equation:

$$\textit{Percent germination}\ (\%) = \frac{\textit{Seeds germinated}}{\textit{total seeds}} *100$$

Statistical Analysis

The experiments were conducted in a completely randomized design. All experiments were conducted with three replicates. The data were subjected to analysis of variance (ANOVA) using SPSS-22 software (SPSS In., Chicago, IL., USA), and means were separated using Fisher's LSD test at 0.05 probability. Statistical significance was determined using a P value of less than 0.05.

Results

Effect of temperature on seed germination

The influence of a constant temperature range for varied incubation periods on seed germination of *Lolium temulentum* L. is shown in Table 1. According to the results of the statistical analysis at the end of the experiment, the effect of different temperatures on the germination of *L. temulentum* seeds was found to be statistically significant. This weed's seeds did not germinate after one day at any of the temperatures examined, nor did they germinate at 35 °C and 40 °C for any period studied. After three days, the first germination was recorded at temperatures of 20 °C, 25 °C, and 30 °C, with germination percentages of 3.33, 13.33, and 1.66 %, respectively. Seeds germinated by 40 % at 15 °C after 7 days, while at a temperature of 10 °C, seeds germinated after 14 days by 91.66% and at 5 °C after 28 days 90.83 %. The maximum germination percentage was 91.66 %, recorded at 10 and 20 °C after 14 days and stayed constant after 28 days. From the results, it was noted that the percentage of germination increases gradually with the length of the incubation period (Table 1).

Table 1. Effect of a constant temperature range for varied incubation periods on seed germination percentage of *Lolium temulentum* L.

Period	Temperature (°C)							
	5 °C	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
1 day	0	0	0	0	0	0	0	0
3 day	0	0	0	3.33	13.33	1.66	0	0
7 day	0	0	40	59.16	44.16	5	0	0
14 day	0	91.66	90.83	91.66	84.16	79.16	0	0
28 day	90.83 a	91.66 a	90.83 a	91.66 a	84.16 b	79.16 c	0 d	0 d

*Treatments with the same letter are not statistically different; P ≤ 0.05

In the germination temperature study for *L. temulentum* seeds, it was determined that the most suitable germination was between 5-20 °C (Table 1 and 2). In the study, it is seen that 35 °C and above stops the seed viability activity. The germination temperature with the highest rate of germination (91,66 %) was determined as 20 °C. However, considering the total germination rates at the end of the experiment, there was no statistical difference between 5, 10, 15 and 20 °C, and they were all in the same group.

Looking at the germination times (T_{10}, T_{25}, T_{50} and T_{90}) of *L. temulentum* in the different temperature, it was determined that the seeds germinated in a minimum of 3 and a maximum of 28 days (Table 2). At temperatures outside of 5 °C, the seeds completed their germination in the first 14 days, and germination continued until the 28th day at 5 °C.

Effect of light on seed germination

The seed of *L. temulentum* did not germinate when incubated in darkness for 14 days. In contrast, it germinated at 32.5 % when incubated in light for 12 hours daily, as shown in Table 3 These findings suggest that seeds of this species may not germinate at greater soil depths. The germination of a seed is affected by light.

Table 3. Effect of lighting condition on seed germination of *Lolium temulentum* L.

Lighting condition	Seed germination (%)
Light	32.5 a
Dark	0 b

*Treatments with the same letter are not statistically different; P ≤ 0.05

Effect of pH on seed germination

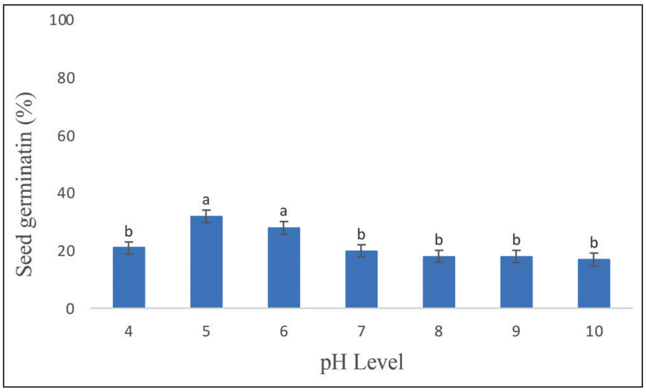
The findings of this study show that there is no significant influence of pH on seed germination of *L. temulentum* at any of the tested levels (Figure 1). This weed has no clear preference for a particular pH level, as shown by the fact that it germinates well throughout the pH range of 4 to 10. Although the highest germination rate is observed at pH 5, there is no statistically significant difference in germination rate between pH5 and pH6 levels (Figure 1). Most weeds have generalist features that enable them to grow in many soil types and habitats, including disturbed and degraded environments.

Table 2. Germination rates and durations of *Lolium temulentum* L. at different temperatures.

Temperature(°C)	G_{max} (%)	T_{10} (day)	T_{25} (day)	T_{50} (day)	T_{90} (day)
5 °C	91.11±1.1b*	28±0a	28±0a	28±0a	28±0a
10 °C	92.22±1.1a	14±0b	14±0b	14±0b	14±0b
15 °C	91.11±1.1b	7±0d	7±0c	14±0b	14±0b
20 °C	87.78±1.1c	7±0d	7±0c	7±0c	14±0b
25 °C	71.11±1.1e	3±0e	7±0c	7±0c	14±0b
30 °C	75.56±2.9d	11.67±2.3c	14±0b	14±0b	14±0b
35 °C	0±0f	0±0f	0±0d	0±0d	0±0c
40 °C	0±0f	0±0f	0±0d	0±0d	0±0c

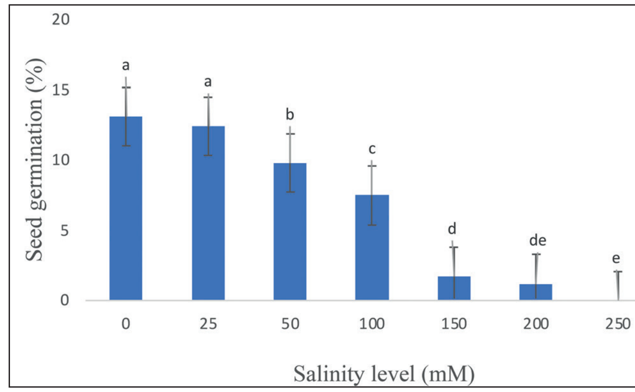
± = Standard deviation values. *Treatments with the same letter are not statistically different; P ≤ 0.05

Figure 1. Effect of different levels of pH on seed germination of *Lolium temulentum* L.



*Treatments with the same letter are not statistically different; $P \leq 0.05$

Figure 2. Effect of different salinity levels on seed germination of *Lolium temulentum* L.



*Treatments with the same letter are not statistically different; $P \leq 0.05$

Effect of salinity on seed germination

Data illustrated in Figure 2 showed significant effect of examined salinity levels on the seed germination of *L. temulentum*. Although the seeds of this weed did not germinate at a salinity level of 250 mM, the seed germination in the control treatment was 13.33 % (Figure 2).

Discussion

The findings of this study indicated that *Lolium temulentum* L. had the highest germination rate (91.66 %) when incubated at 10 °C and 20 °C for 14 days and that this percentage remained constant even after extending the incubation time. Seeds of this species may also germinate at low temperatures if the incubation period is prolonged. The seeds of this weed, on the other hand, did not germinate at temperatures of 35 °C or above, even after the longest incubation time tested, indicating that germination may be delayed, or the seed may become dormant at elevated temperatures. In the study conducted by Lu et al., (2006), the germination of Crofton weed (*Eupatorium adenophorum* Spreng.) seeds was examined by applying different factors. Although the seeds germinated at temperatures between 10 °C and 30 °C applied to the seeds, the optimum germination value was obtained at 25 °C.

Temperature is the most critical factor influencing germination, as it affects germination in three ways: moisture, hormone production, and enzyme activity (Finch-Savage & Leubner-Metzger, 2006). Seed germination needs a specific quantity of moisture; in a hot climate, moisture levels may decline, impacting germination (Baskin & Baskin, 2014). Temperature has an important effect on the weed species adaptability. Temperature of soil is a main ecological aspect that influences weed seed germination and development. The emergence rate of weeds is strictly associated with temperatures of soil (Singh & Singh., 2009). Temperature adjusts germination by eliminating dormancy (Benech-Arnold et al., 1990).

L. temulentum seeds did not germinate when incubated in darkness for 14 days. In contrast, it germinated at 32.5 % when incubated in light for 12 hours daily. Positively photoblastic seeds are driven to germinate by light, whereas negatively photoblastic seeds are those that are inhibited by light. The phytochrome-regulated synthesis of the plant hormone gibberellin modulates the response to light (Baskin & Baskin, 2014). The seeds were positively photoblastic, meaning that light encouraged germination while darkness inhibited it (Chen et al., 2013).

According to result of these experiments seeds of *L. temulentum* germinate across a wide pH range, indicating that this species is pH-tolerant and may survive in various soils. Florentine et al. (2016), Hao et al. (2017) and Humphries et al. (2018), who investigated diverse weed species, found comparable results. Furthermore, Perez-Fernandez et al. (2006) suggested that the germination processes in this species are not pH-dependent. Since Large Crabgrass (*Digitaria sanguinalis* (L.) Scop.) is a common weed in the fields, the effect of soil pH on Large Crabgrass was examined by Pierce et al., (1999). The seeds were planted in a loamy sand soil amended with calcium carbonate ($CaCO_3$) or magnesium carbonate ($MgCO_3$), which creates a soil pH range of 4.8 to 7.8. It was observed that the germination of Large Crabgrass seeds was not affected by the pH change when the soil was changed with $CaCO_3$, while it was observed that the seed germination decreased with the increasing pH value when it was replaced with $MgCO_3$. In another study conducted by Lu et al., (2006), the pH value of Crofton weed (*E. adenophorum*) germination was found between 5 and 7.

Singh & Singh (2009) studies were conducted on the effect pH and light exposure on seed germination of Brazil pusley, common ragweed, Florida beggarweed, hairy beggarticks, ivyleaf morningglory, Johnson grass, prickly sida, redroot pigweed, sicklepod, strangler vine, tall morningglory and yellow nutsedge. In the study, it was observed by the examiners that the species in terms of pH, it was observed that the pH range of 5 to 11 did not adversely affect the germination of weed species.

The change in soil pH level also changes the weed control efficiency. Chadha et al. (2019) stated that the decrease in control of *Abutilon theophrasti* Medicus and *Setaria faberi* Herrm. decreased when pH was raised from 5 to 6; however, control of *Amaranthus retroflexus* L. *and Chenopodium album* L. only occurred at the highest soil pH tested.

Outcomes of these research enounce that *L. temulentum* seeds could germinate in high saline circumstances, which can be an important issue for weed species allowing it to settle saline areas. Salinity is a significant abiotic stress factor for crop production globally. Soils with more than 100 mM NaCl are evaluated to have high salt substances. Crop manufacture might be affected through the soil salinity in addition to weed competition. Like *L. temulentum, Hibiscus tridactylites* Lindley (Chauhan, 2016) and *Mimosa invisa* Mart. ex Colla (Chauhan & Johnson, 2008) seeds germinated at very high salt concentrations. In another study conducted by Lu et al., (2006) on Crofton weed (*E. adenophorum*), the germination status of the weed changes at different salt concentrations applied. Crofton weed seeds germinated at values below 100 mM NaCl, but not at 300 mM NaCl. Swallowwort (*Cynanchum acutum* L.) seeds, another weed applied with different salt concentrations, did not germinate at 300 mM NaCl, but 12% germination was observed at 200 mM NaCl (Pahlevani et al., 2008).

High salinity may affect embryo viability primarily through hormonal changes, particularly abscisic acid (ABA) synthesis, which is known to induce or maintain seed dormancy, resulting in a lower germination rate and a longer germination time, or through other effects such as reduced water absorption and cell damage (Thiam et al., 2013; Ibrahim, 2016). The findings of this study confirm that the seeds of this species are salinity tolerant since they germinate even a small percentage at an extremely high salinity level (200 mM).

Conclusion

Weeds are found ubiquitously in almost every environment globally, and an important agricultural weed, *Lolium temulentum* L., grew and was challenging to manage in different climates conditions. Therefore, it is a weed that must be managed. In herbology studies carried out worldwide, weed control is generally carried out by looking at the biology and physiology of weeds. The study was carried out to understand how weeds respond to light, temperature, pH, salinity and when these germination processes are activated throughout the stress.

According to the findings of this study, the optimum germination temperature for seeds of this species is between 10 and 20 degrees Celsius, and if there are temperatures of 20 °C and above, they can start to germinate within 3 days. Seeds of this species take 28 days to germinate at temperatures around 5 °C, and cold weather slows germination. According to the study, the seeds of this species were also positively photoblastic, indicating that they may not germinate in darkness. Furthermore, there was no influence of pH on the weed's seed germination, showing that it may germinate and grow in a range of soils. According to seed germination studies, this species is salt resistant and can tolerate salinity of up to 200 mM NaCl.

The data obtained from the study show that *L. temulentum* can germinate and spread even in extreme environments such as temperature, pH and salinity. This shows that this species has the potential to spread rapidly and cause damage in agricultural areas. Therefore, it is necessary to take measures to prevent the spread of this species. In preventing the spread of *L. temulentum*, clean production materials and equipment to be used in the field are clean, animal manures that may contaminate weed seeds are not used, if irrigation is to be done, attention should be paid not to let weed seeds come to the field with irrigation water, and plant rotation is among the important issues.

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