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Germination and Seedling Growth Responses of *Zygophyllum fabago, Salsola kali* L. and *Atriplex canescens* to PEG-Induced Drought Stress

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Abstract: In arid and semi-arid regions, planting drought-tolerant species is the most useful strategy in the reclamation of degraded soils. In the present study, we evaluated the effect of simulated drought by polyethylene glycol (PEG-6000) on seed germination and seedling growth of three desert plants such as *Atriplex canescens*, *Salsola kali* and *Zygophyllum fabago*. Seeds were subjected to water stress to drought stress by PEG at five stress levels (0, −1, −4, −8, −12, −14 bars). Germination of *Z. fabago* was completely inhibited at an osmotic potential of −8, −10 and −12 bars and the germination of *A. canescens* was inhibited only at −14 bar. In contrast, *S. kali* responded positively to high levels of stress and our results showed the highest final germination percent (71.75, 54 and 18.25%) under three-drought stress −8, −12 and −14 bars, respectively. In addition, increasing PEG concentration adversely affected the germination rate and seedling vigor index as well as the root and shoot length of species. Under high stress levels, *S. kali* achieved a higher germination rate and seedling vigor index compared to *Z. fabago* and *A. canescens*. Among species, *S. kali* was the only one able to develop roots and shoots at −14 bar. Therefore, *S. kali* could be considered as a promising plant for the rehabilitation of degraded soils at risk of desertification.

Keywords: *Atriplex canescens*; *Salsola kali*; *Zygophyllum fabago*; germination; osmotic potential; tolerant

1. Introduction

Among abiotic stresses, drought stress is one of the most devastating factors that impact and reduce crop productivity worldwide [1,2]. Drought stress influences many important morphological, physiological, and biochemical responses such as cell turgor, stomatal activity [3–5], photosynthesis rate [6,7], and alter vegetative growth, biomass partition in plats [8–10]. Exposure to this stress influences almost every developmental stage of the plant such as germination, seedling growth and flowering [11–13]. Seed germination is a critical stage in plant life, which affects the successful establishment of seedlings and subsequent growth. In this stage, plants are extremely sensitive to drought stress [14].

One method for studying the impact of water deficit stress on germination is to induce stress conditions using artificial solutions to prepare different water potentials [15,16]. PEG-6000 is a polymer and considered a better chemical than others to generate drought stress without any toxic effect [17,18]. This compound has been used mostly in plant water deficit studies to stimulate dehydration by declining water potential [19].

Atriplex canescens (Pursh.) Nutt a perennial semi ever-green shrub from Chenopodiaceae family [20,21]. The plants of the genus of *Salsola* and *Atriplex* are widely distributed in the hypersaline, arid and semi-arid areas of the world. These species, which can be used extensively in re-vegetation projects for erosion control, have sand fixation due to their tolerance to drought, salt and heat stress [22]. *Zygophyllum fabago* L., commonly known as a Syrian bean caper, is widespread in Iran, Pakistan, Afghanistan, Iraq, Arabia, North Africa, Spain, France, Italy and Turkey. The Iranian flora consists of nine species of *Zygophyllum* growing in different areas of the country [23,24]. Seedling of *Z. fabago* has a strong drought tolerance. Thus, this plant is used for recovery, improvement and protection of soil in desert ecosystems. Several studies have evaluated the effect of soil water potential (induced by PEG-6000) on the germination of some desert plants [25–27]. The authors of [28] reported the water stress stimulated by PEG on *Atriplex undulate*. Results revealed that *A. undulata* without bracteoles, presented a 50% reduction in germination percentage under −0.7 MPa, compared to the non-stressed control.

Ma and colleagues [29] studied the germination of *Salsola ferganica* and showed that this plant can germinate more than 60% at −500 KPa OP, which suggests that *S. ferganica* is drought-tolerant.

To the best of our knowledge, there is no information available on the effects of simulated drought by polyethylene glycol (PEG-6000) on seed germination of three desert species. The results of the studies may be used in the identification of appropriate species for the rehabilitation of degraded lands in dry and semi-dry areas. Therefore, the objective of this experiment was to evaluate the impact of water stress based on PEG on seed germination and seedling growth of *Z. fabago*, *S. kali* and *A. canescens*.

2. Material and Methods

2.1. Plant Materials

Salsola kali and *A. canescens* seeds were collected during autumn 2017 from arid and semi-arid lands at Zanjan University Research Farm, Zanjan, Iran (36°41′ N and 48°23′ E; altitude 1634 m). *Z. fabago* harvested in summer 2017 from Zanjan province, Tarom (36°52′ N and 48°55′ E). The seeds were harvested manually close to the stage of physiological maturity (seed ripening) then stored at room temperatures (15–19 °C, 20–35% humidity) until the start of the experiment. The surface of seeds was cleaned with sodium hypochlorite (10%) and finally soaked eight times with distilled water. Application of 5 levels of drought stresses with different osmotic potentials of −1, −4, −8, −12 and −14 bars were prepared as described by [30]. Distilled water was used as a control treatment. Twenty-five seeds were selected and placed in two-fold filter papers moistened with 10 mL of deionized water or PEG-6000 solution. The Petri dishes were sealed with parafilm to prevent evaporation and placed accordingly to a completely randomized design in an incubator at 25 ± 1 °C in the dark. Three replicates were used for each treatment. The number of germinated seeds was counted daily up to 12 days, and each seed was considered to have germinated when there is a protrusion of the radical [31].

Germination percentage was estimated by using the following equation: GP = 100 (NG/NT), where NG is germinated seeds, NT is total seeds [32].

Germination rate was obtained as follows:

$$R_s = \sum_{i=1}^{n} \frac{S_i}{D_i} \quad (1)$$

where: S_i = number of germinated seed per each calculation; D_i = number of day until calculation; n = number of calculation [32].

Seedling vigor index was computed with equation (s + r) G, where s and r are the shoot length (in cm) and root length (in cm), respectively and G is the percentage of germination [33]. The root and shoot length of all germinated seeds were measured using a ruler 12 days after germination (end of the experiment).

Mean daily germination (MDG): mean daily germination is calculated by the following equation [34]:

$$\text{MDG} = \text{FGP/d} \quad (2)$$

In this equation, FGP is the final germination percentage (viability) and d is the number of days to achieve final germination (experiment duration).

2.2. Data Analysis

The data were statistically analyzed using SAS Software (Version 9.1, SAS Institute Inc., Cary, NC, USA). The difference between the means was compared to Duncan's test ($p < 0.05$). All data were expressed as mean ± standard error (SE). All graphs drown using the Sigma Plot 13.0 software.

3. Results

3.1. Germination Percentage

The Germination percentage of species were impacted greatly by increasing drought stress. Figure 1 shows that for all species, increasing stress levels resulted in a significant reduction in germination percentage. The final germination percentage of the control (0 bar) recorded 98.5, 57.25 and 53% for *S. kali*, *A. canescens* and *Z. fabago*, respectively. *S. kali* showed the highest final germination percent (71.75, 54 and 18.25%) under three-drought stress −8, −12 and −14 bars, respectively, while *Z. fabago* had a final germination percentage of zero at these respective stress levels. In comparison, germination percentage of *A. canescens* at the same PEG concentrations were (14.5%, 5% and 0) respectively. Among species, *S. kali* recorded higher final germination than *A. canescens* and *Z. fabago* at different concentrations of PEG.

No germination appeared at −14 bar for *A. canescens* and *Z. fabago*, while *S. kali* had a different degree of germination percentage at the same concentration. *S. kali* seeds exposed to 0, −1 and −4 bars showed germination higher than 65% on the 2nd day. On the contrary, *Z. fabago* did not germinate under similar conditions. At 4 days after treatment (Figure 1), the germination of *Z. fabago* was all inhibited under −4, −8, −12 and −14 bars, while *A. canescens* presented 25, 13.75, 4% and 0 respectively and *S. kali* showed higher values in comparison to *A. canescens*. In the absence and presence of drought stress, *S. kali* had the highest germination percentage compared to *Z. fabago and A. canescens* on the 6th day. These findings revealed that, in terms of germination percent, *S. kali* was the best performing species under severe water stress conditions, and *Z. fabago* was the most sensitive. *A. canescens* presented an intermediate response.

3.2. Mean Germination Time

The mean germination time of three plant species reduced by using PEG-6000 compared with the control treatment. However, by increasing stress levels, different responses among the studied species were observed. The seeds of *S. kali* showed the highest mean germination time (8.2, 8.14, 7.95 and 7.81 days) at the osmotic potential of 0, −1, −4 and −8 bars, respectively while the lowest ones (1.52 days) were recorded for *S. kali* at −14 bar. The mean germination of time of *A. canescens* was 4.77 days at 0 bar, and reduced sharply to 1.20 at −8 bar. The mean germination of time of *Z. fabago* was impacted greatly by increasing drought stress. Osmotic potential of −4, −8, −12 and −14 bars decreased the mean germination of time of *Z. fabago* to zero (Figure 1).

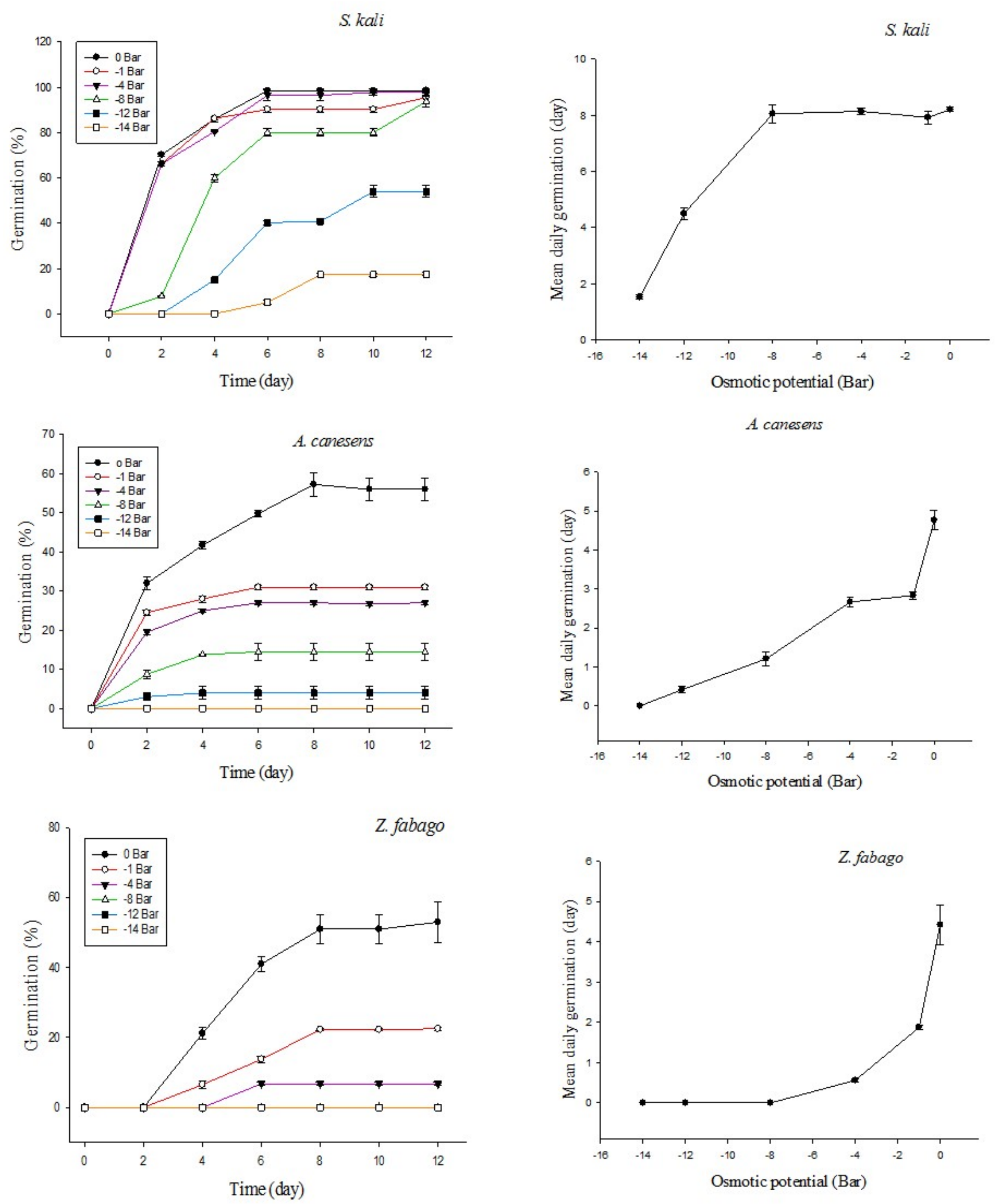

Figure 1. Germination (%) of *S. kali*, *A. canescens* and *Z. fabago* under different polyethylene glycol (PEG-6000) concentrations at different times (**left**) and mean daily germination of *S. kali*, *A. canescens* and *Z. fabago* under different PEG-6000 concentrations (**right**). Data represent means (n = 4) ± SE.

3.3. Germination Rate

Germination rate under water stress was reduced significantly compared with that of the control (0 Bar). All three species exhibited a decreasing trend in germination rate, as the stress level increased. Among three species, *S. kali* was the fastest in germination at all concentrations. In the case of *Z. fabago*, water potential of −8, −12 and −14 bar decreased germination rate by 100% compared to the non-stressed control. In *A. canescens,* treatment with −4, −8 and −12 bars significantly decreased the germination rate by 59, 78 and 93% respectively, compared to the non-stressed control (Figure 2).

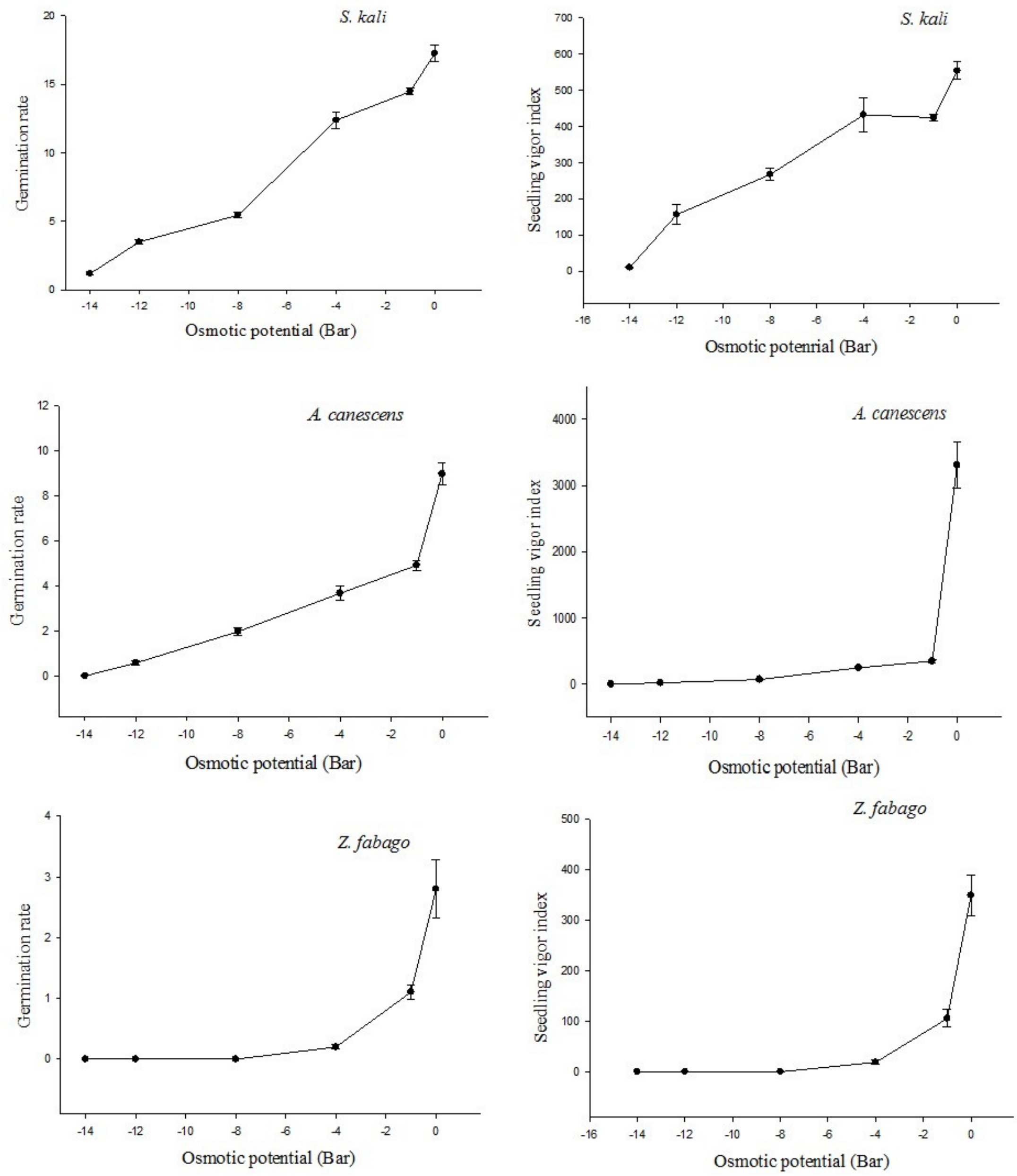

Figure 2. Germination rate (**left**) and seedling vigor index (**right**) of *S. kali*, *A. canescens* and *Z. fabago* under different PEG-6000 concentrations. Data represent means (n = 4) ± SE.

3.4. Seedling Vigor Index

In the present study, drought stress induces an inhibitory effect on seedling vigor index. With increasing PEG-6000 concentrations, the vigor index of all three species declined. This decline was more obvious in *A. canescens* as compared with *S. kali* and *Z. fabago* and the greatest vigor reduction was found in these species at −1 bar. The maximum values of the vigor index were achieved in three studied species in absence of PEG. Under water stress conditions, the seedling vigor of *S. kali* at −8, −12 and −14 bars were 267, 155 and 9.84, respectively. In contrast, *Z. fabago* presented zero under the same concentrations (Figure 2).

3.5. Root and Shoot Length

According to our results, the root and shoot lengths of three species were significantly affected by drought stress. However, by increasing stress level, different responses among the investigated species weree found. In *Z. fabago*, root growth was severely inhibited by 100% at −8, −12 and −14 bars while in *S. kali* root length increased by 34% at −4 bar compared to -1 bar and the maximum root length (5.47 cm) was identified in *A. canescens* at (−1 bar). In *A. canescens*, seeds root length increased by 54% at -1 bar compared to the control treatment (Figure 3).

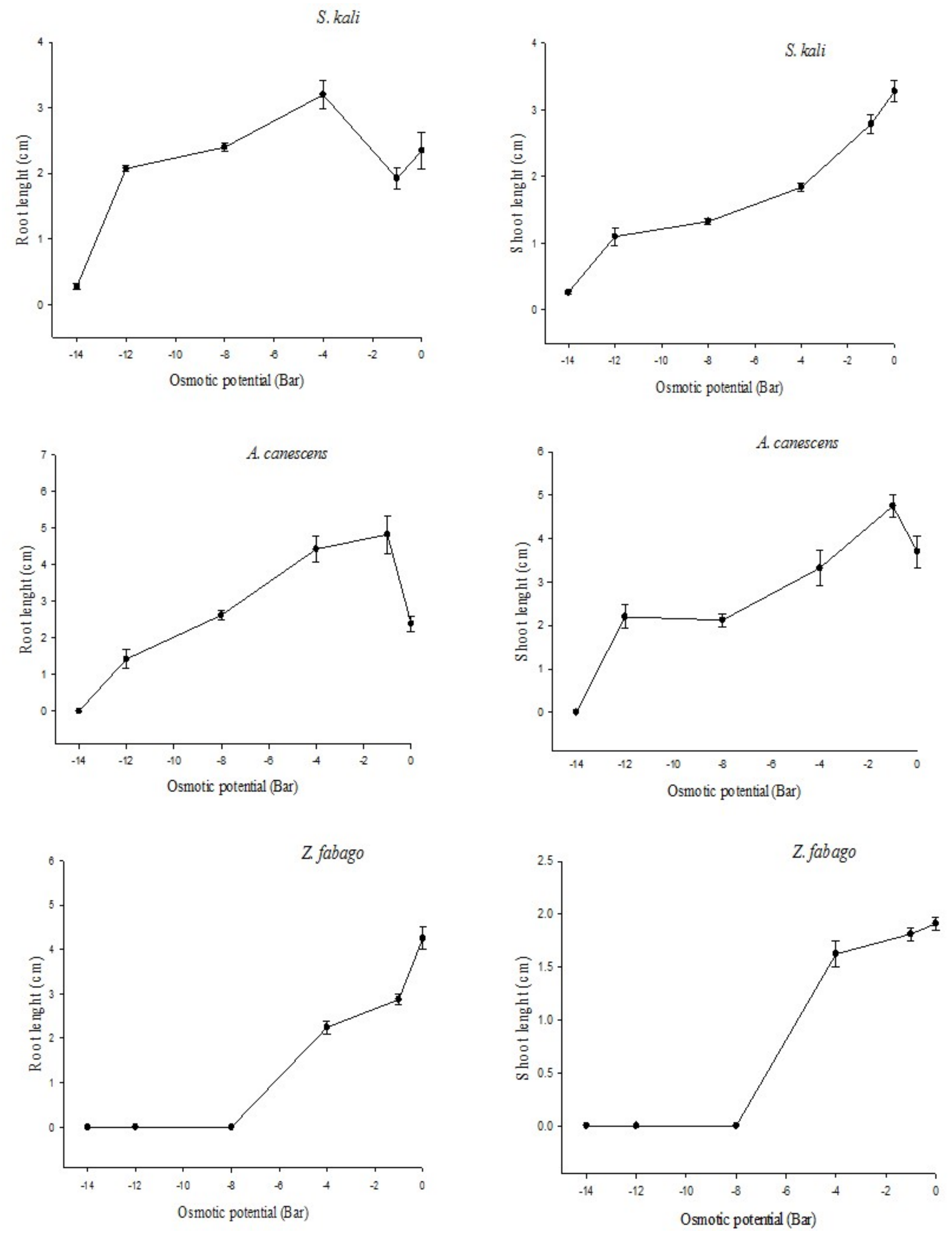

Figure 3. Root (**left**) and shoot length (**right**) of *S. kali*, *A. canescens* and *Z. fabago* under different PEG-6000 concentrations. Data represent means (n = 4) ± SE.

In absence of stress (control), *A. canescens* showed the highest shoot length (3.4 cm) followed by *S. kali* (3.27 cm), while *Z. fabago* presented the lowest one (1.8 cm) (Figure 3). At −1 bar, the highest shoot length (4.75 cm) was recorded in *A. canescens* and in this species, shoot length increased by 28% at −1 bar compared with non-stress control. Among species, *S. kali* was the only one able to develop shoots at −14 bar and *Z. fabago* showed the lowest shoot length (1.6 cm) at −14 bar, and failed to germinate at −8, −12 and −14 bars (Figure 3).

4. Discussion

Drought is a major abiotic stress that seriously decreases crop productivity in arid and semi-arid environments [35–39]. It has been reported that PEG decreases the water potential of osmotic solutions and thus can be used for drought stress studies. In our experiment, increasing PEG concentration adversely impacted seed germination and seedling growth of species, as a consequence of decreased water uptake [40]. Our results highlighted that *S. kali* seeds germinated well (>50%) at −12 bar. As a result, *S. kali* was tolerant to moistures stress at germination and thus is able to germinate in dryer areas. Under low water potential conditions caused by osmotic stresses like drought or salinity, some plants can efficiently accumulate various substances for osmotic adjustment (OA), and thus enhance the water absorption and retention capacity, which plays an important role in plants adapting to water-deficient conditions [41–43]. The adaptations of this species to drought conditions make it a particularly invaluable species for use in the reclamation of degraded soils at risk of desertification [44]. In this study, there was no germination of *Z. fabago* seeds under −8, −12 and −14 bars, suggesting this species was not tolerant to drought stress at germination. In contrast, the germination of *A. canescens* was inhibited only at −14 bar. These results are in accordance with [45], who found that osmotic potential of −10 and −12 bars completely inhibited seed germination in *Brassica juncea* but in the shrub species (*Artemisia sphaerocephala*), tolerated only −1.5 MPa PEG [27]. Reduction in seed germination percentage by water stress may be related to lower infusibility of water through the seed coat and initial water absorption by seeds under stress condition [46–48].

Drought stress influenced the germination rate and mean time for germination (MTG) in all species. Notably, *S. kali* had higher and quicker germination than *A. canescens* and *Z. fabago* at all concentrations but the germination rate of *Z. fabago* seeds was inhibited at osmotic potential of −8, −12 and −14 bars. These results are in agreement with previous studies where germination rate decreased in *Agropyron elongatum*, *Agropyron desertourm* and *Secale montanum* when exposed to a high concentration of PEG-6000 [49]. The faster germination at lower osmotic potentials has been considered a strategy for seedling establishment, which can decline competition, especially in years that receive less than average rainfalls [50,51].

The results of seedling vigor index showed that with decreasing water potential, the vigor values decreased, while *S. kali* achieved high values of vigor index under high PEG concentration. Seed vigor, an important index of seed quality, evaluates the potential for fast and uniform emergence of plants. The early vigor of seedling with good development can be used as a beneficial trait of interest for the selection of tolerant species [52,53]. Siddique and colleagues [54] suggested that plants with a higher vigor index could improve crop water use efficiency.

Root length is considered an important criterion for the selection of drought-resistant species [55,56]. The root length reduction in plants under different PEG-6000 concentrations may be attributed to a reduced cellular division and elongation during germination [57]. Drought conditions decrease the uptake of nutrients by the plants due to limited soil moisture, leading to diminished stem length [58]. Reduced shoot and root lengths are in agreement with results reported by [59] in soybean genotypes.

5. Conclusions

This study revealed that, in terms of germination percent, *S. kali* was the best performing species under severe water stress conditions, and *Z. fabago* was the most sensitive. *A. canescens* presented an intermediate response. Therefore, *S. kali* could be considered as a promising plant for the rehabilitation of degraded soils at risk of desertification.

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