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Proceeding Paper

Effects of Salinity and Drought Stress on Seed Germination of Common Purslane (*Portulaca oleracea*) †

Anh Cong Pham, Tuan Chau Vo, Hoang Duc Vu and Dan Quang Tran *

Faculty of Biology and Environmental Science, University of Education and Science—The University of Danang, The University of Da Nang, Da Nang City, Vietnam; boconganhptt123@gmail.com (A.C.P.); vctuan@ued.udn.vn (T.C.V.); vdhoang@ued.udn.vn (H.D.V.)
* Correspondence: tqdan@ued.udn.vn; Tel.: (+084)-702651086
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Abstract: Common purslane (*Portulaca oleracea*) is a halophyte, consumed not only as an edible vegetable but also as a traditional medicine. This plant can adapt to extreme salinity and drought conditions and their effects on plant growth, yield and quality were elucidated; but the effects on seed germination was still unclear. Seed germination is an important stage for establishing *P. oleracea* seedlings that contribute to plant yield and productivity. Thus, the objective of present study was to examine individual effects of salinity and drought stress at different levels on characteristics of seed germination, which the seeds were suffered from −0.22, −0.45, −0.89, −1.78 MPa osmotic stresses induced by PEG, or from 50, 100, 200, 400 mM NaCl stresses with equivalent osmotic pressures. The seeds sown in petri dishes containing filter paper layers flooded with NaCl or PEG-6000 solutions for the treatments, and germination parameters were determined daily for 15 days. Data showed germination percentage (GP), germination rate (GR), germination energy (GE), and mean germination time (MGT) were significantly decreased with increasing levels of salt and osmotic stresses, suggesting that the salinity and drought stress reduced germination capability of seeds. Moreover, the seeds were maintained germination with PEG osmotic pressure above −0.22 MPa, but not with NaCl concentration greater than 50 mM that induced a similar osmotic pressure, suggesting that the ion toxicity effect on the seed germination might be higher than the osmotic effect.

Keywords: salinity stress; drought stress; seed germination; *Portulaca oleracea*

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1. Introduction

Salinity and drought have been becoming worldwide major threats to agricultural production. Salinity and drought stress interrupt functional cellular processes such as water absorption, photosynthesis, nutrient uptake and metabolisms, leading to inhibition of growth and development of plants [1]. Plants face up with osmotic stress under water deficiency, which caused by high osmotic pressure of extracellular environment [2]. Meanwhile, under high salinity condition mainly caused by NaCl, plants might suffer from both osmotic stress and ion toxicity of Na^+ and Cl^- [3].

Seed germination is the initial stage in plant's life cycle and plays an important to yield of seed-propagated crops, but it is sensitive to salinity and drought stress. During germination, water imbibition is the first step that required to activate enzymes and promote development of embryo [4]. As a result, osmotic stress-inducing factors would reduce cell's water uptake ability, thus inhibit seed germination [5]. Besides, the present of Na^+ and Cl^- ions at certain concentration might impose toxicity on embryonic metabolisms and seedling formation [3]. However, inhibition level of these stresses to germination depends on physiology status of seeds and plant species. Thus, understanding germination capability and characteristics of seeds under unfavorable condition is significant for strategic development of agricultural practices.

Common purslane (*Portulaca oleracea*) is an annual herbaceous creeping plants, commonly cultivated around the world [6]. This is a potential vegetable and herbal crop owing to its nutritional and medicinal value. The leaves are rich in omega-3 fatty acids, a-linolenic acid, a-tocopherol, ascorbic acid, beta—carotene and glutathione [6]. Studies with in vitro models showed that the whole plant extract has high antioxidant activity compared to other vegetables due to the high level accumulation of antioxidant compounds. In addition, *P. oleracea* are able to adapt to adverse environmental conditions such as drought and salinity. Growth responses of *P. oleracea* plants under salinity and drought stresses have been elucidated in previous studies. It also has demonstrated the ability to maintain growth and enhance synthesis of bioactive substances under severe water deficient and saline conditions [6,7]. Although the plant is mainly propagated and regenerated by seed, the effects of salinity and drought stresses on the germination of *P. oleracea* seeds have not been investigated so far.

The objective of present study was to examine effects of salinity and drought stress at different levels on germination of *P. oleracea* seeds and evaluate the influence level of these two stress factors. For this purpose, seeds were sown under saline conditions induced by NaCl at concentrations of 50, 100, 200, and 400 mM, and physiological drought conditions with osmotic pressure of −0.22, −0.45, −0.89, and −1.78 MPa induced by PEG-6000. The osmotic pressures were respectively equivalent between two salt and osmotic stress levels. PEG-6000 is a water-soluble polymer that was commonly used to simulate effects of drought stress on seed germination [8].

2. Material and Methods

2.1. Seed Collection

P. oleracea plants were grown in a greenhouse placed in University of Education and Science—The University od Danang for blossoming. Fruits were collected the plants after the flower withering, and stored in plastic boxes under well-ventilated conditions, at room temperature till fruit opening for seed collection.

2.2. Seed Germination and Treatments

The seeds were sown according to a method as described by Dan et al., 2022 [9]. The A seed sterilization procedure was carried out to eliminate microorganism contamination prior to sowing. After soaking with Nano Kito 2.6SL fungicide solution prepared according to the manufacturer’s instruction, the seeds were treated with 0.1% $HgCl_2$ solution for 10 min and rinsed 3 times with sterile distilled water. About 50 decontaminated seeds were spread in a 9 cm-diameter petri dish containing two filter paper layers flood with 5 mL of treatment solutions. For the salt treatment, the solutions were prepared with 50, 100, 200, and 400 mM NaCl; and for the drought treatment, the solutions were prepared with amount of PEG-6000 that produce osmotic pressures at −0.22, −0.45, −0.89, and -1.78 MPa. The seed were also sown with only water that considered as control. The petri dishes were placed in a growth room for 15 days with set conditions such as temperature at 25^0 C and a photoperiod for 14 h/day with light intensity at 2.000 lux. Water was added to the petri dish weekly to maintain the wetness of the filter paper.

2.3. Determination of Germination Parameters

The seeds were considered as germinated when shoot and root tips appeared. Germinated seeds were counted daily during 15 days. Germination parameters such as germination percentage (GP) (%), germination rate (GR) (%/day), germination energy (GE) at day 7 after sowing (%) and mean germination time (MGT) (days) were calculated as described by Kader et al. (2005) [10] and Shiade et al. (2020) [2].

2.4. Experimental Design and Data Analysis

Seed germination experiments were randomly carried out with 100 seeds and repeated three times ($n = 3$) for each stress level. Analysis of descriptive statistics was applied to germination parameter, and the significant difference between the stress levels were compared following to Duncan's test with significance level $\alpha = 0.05$ using R software.

3. Results and Discussions

3.1. Effects of Salinity Stress on Germination of P. oleracea Seed

Data showed that the salt stress levels have significant effects on germination of *P. oleracea* seeds. The GP of seeds were decreased with increasing salt stress levels. In control condition (without salt), the seeds began to germinate two days after sowing with GP up to 66.22%; and it was increased in the following days and reached a maximum value of 95.93% on the day 9 of treatment time (Figures 1a and 2a). However, presence of NaCl in the treatment solutions inhibited germination that the seeds could germinate only in 50 mM NaCl solution at only the day 5 of treatment with a low value of GP. The GP of salt-treated seeds was only 4.79% at the first germination time that declined 91.14% compared to the seeds under the control. Shoots and roots emerged under this salt treatment showed no morphological difference from those in the control. Remarkably, the seeds did not germinate with 100–400 mM salt levels during the observation time (Figure 1a).

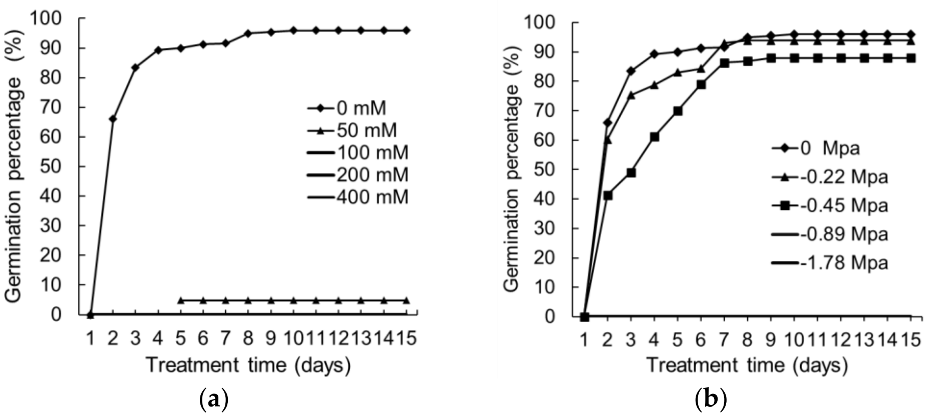

Figure 1. Germination percentage of *P. oleracea* seeds under different stress levels during 15 days after the onset of treatment. (**a**), salinity stress; (**b**) PEG-induced osmotic stress.

Besides germination percentage, other germination parameters were also significantly decreased with 50 mM NaCl treatment, in comparison to that in the control ($p < 0.05$). The GR and MGT of seeds under the control were 41.20%/day and 2.72 days, respectively; however, the GR was reduced to 0.96%/day and the MGT was increased to 5 days when the seeds were treated with 50 mM NaCl concentration (Figure 2b,c). This suggested that salinity stress delayed seed germination time. In addition, the GE of seeds was also significantly decreased with the salt treatment compared to the control, from 91.55% in the control to 4.79% in the salt-stressed seeds, suggesting that salt stress reduced the germination capability of seeds (Figure 2d).

Previous studies have demonstrated that an increase in salinity level seriously impacted seed germination in various plant species such as *Vigna radiate* L., *Lens culinaris*, *Elymus junceus*, Sorghum species, and pearl millet (*Pennisetum glaucum*) [11]. Salinity can reduce seed germination capability or prolong germination time because of reduced water uptake and toxicity of Na^+ and Cl^- ions caused by salt, which lead to limited hydrolysis of nutrients required for energy supplies of seed germination [12]. It is demonstrated that *P. oleracea* was able to tolerate extreme saline condition [7], but the results in the present showed that the germination of seeds was sensitive to salinity. The seed germination was inhibited under salt stress. This result was also similar with case of *Lathyrus sativus* L. that saline stress reduced seed germination capability and delayed germination process [4].

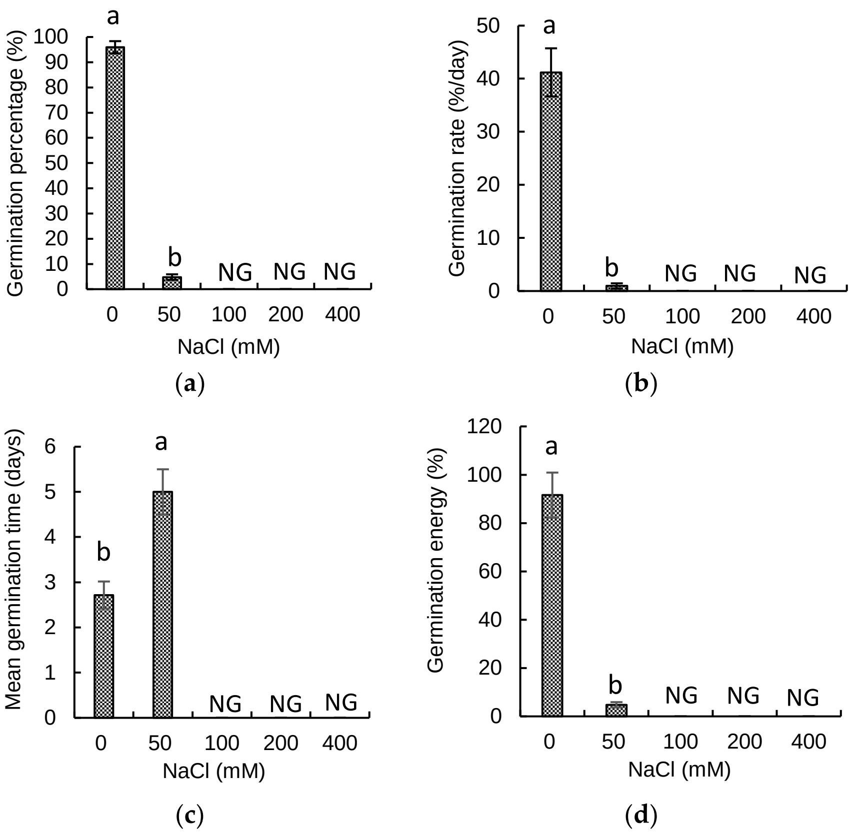

Figure 2. Germination parameters of seeds under salt stress. (**a**), germination percentage, (**b**), germination rate; (**c**), mean germination time; (**d**), germination energy. Letters indicates statistically significant differences between the treatment levels; NG—not germinated.

3.2. Effects of Drought Stress on Germination of P. oleracea Seed

Data showed that the germination of *P. oleracea* seeds was also negative affected by PEG-induced osmotic stress that the seed germination capability were decreased with increasing osmotic stress level. Similar to the control, the seeds were initiated to germinate at PEG concentrations induced −0.22 MPa and −0.45 MPa osmotic pressures at the day 2 of the treatment, but GP was significantly decreased to 60.24% and 41.32%, respectively (Figure 1b). The GP was continued to increase at following days and reached respectively maximum values 93.83% and 87,96% on the day 9 of treatment time, which were decreased 2.10% and 7.07% compared to the control (Figure 3a). The GP was significantly decreased at −0.45 MPa osmotic pressures ($p < 0.05$). The seed germination was inhibited with higher than −0.45 MPa osmotic pressure (Figure 3a). Remarkably, the seeds were maintained germination at −0.22 MPa osmotic pressure with a higher GP than that of 50 mM NaCl treatment, but the osmotic pressure was equivalent (Figure 1). This result suggested that the ion toxicity effect on seed germination under salinity was higher than that of osmotic stress, which caused a more serious impact of salinity on the seed germination.

In addition, the result showed that germination parameters such as GR was also significantly decreased to 30.77%/day, and MGT was significantly increased to 3.77 days with the treatment of −0.45 MPa osmotic pressure ($p < 0.05$) (Figure 3b,c), suggesting that osmotic stress delayed seed germination time. A significant decrease of GE was also observed at −0.45 MPa osmotic pressure (Figure 3d), suggesting that osmotic stress reduced the seed germination capability.

PEG molecules affect germination through limiting the penetration of water molecules into plant tissues, causing physiological drought [12]. Previous stuides demonstrated that osmotic stress caused by PEG led to a decrease in the hydrolysis of seeds, thus a decline in seed germination [13]. The results in the present study were also consistent with previous studies that showed reduced seed germination capability of *Agropyron elongatum*, *Agropyron desertourm* and *Secale montanum* when exposed to high levels of PEG [14].

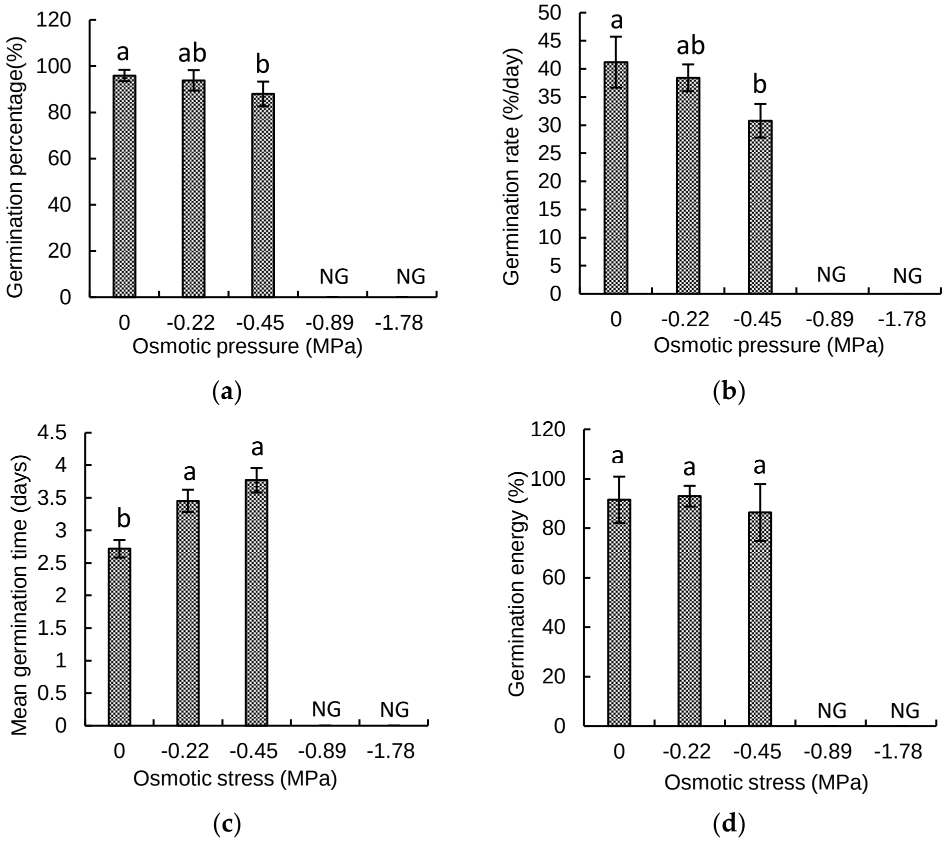

Figure 3. Germination parameters of seeds under osmotic stress. (**a**), germination percentage; (**b**), germination rate; (**c**), mean germination time; (**d**), germination energy. Letters indicates statistically significant differences between the treatment levels; NG—not germinated.

4. Conclusions

In the present study, the results indicated that salinity and drought stress both negatively affected on germination capability of *P. oleracea* seeds. The seeds were only germinated under salinity level not greater than 50 mM with a low germination capacity, and the germination was inhibited with higher salinity levels. Meanwhile, the seeds were germinated under osmotic stress at −0.22 and −0.45 MPa with a significant decrease of germination capability at later level. The seed germination was inhibited with higher osmotic stress levels. The salinity and osmotic stress inhibited germination capability and prolonged seed germination time. Moreover, the ion toxicity effect on seed germination under salinity was higher than that of osmotic stress, which caused a more serious impact of salinity on the germination of *P. oleracea* seeds.

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