Effects of Drought, Salinity, and Acid Stress on Seed Germination of Medicinal Plants Asclepias and Amaranthus: Postprint

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Abstract

Using the medicinal plants Asclepias curassavica L. and Amaranthus as experimental materials, polyethylene glycol (PEG-6000), NaCl, and pH gradient solutions were employed to simulate drought, salt, and acid stress environments, aiming to investigate the response of seed germination in the two plant species to environmental stresses and to provide a theoretical basis for exploring their introduction and cultivation, as well as high-quality medicinal material production, in arid and saline-alkali regions. The results demonstrated that different concentrations of PEG and NaCl significantly inhibited seed germination in both Asclepias curassavica and Amaranthus, with germination rates and germination indices decreasing as concentrations increased. Rehydration treatment of ungerminated seeds after 14 days of stress induced rapid compensatory germination. TTC staining of seeds that remained ungerminated after rehydration revealed that they maintained viability, with mean proportions of viable seeds for the two medicinal plants being 84% and 90%, respectively, which were significantly higher than the control or not significantly reduced. Within a pH range of 3-6, germination rates of Asclepias curassavica and Amaranthus seeds exceeded 57% and 83%, respectively. Rehydration germination of ungerminated seeds and TTC staining results indicated that the stress treatments caused approximately 10% mortality in Asclepias curassavica seeds and 15% in Amaranthus seeds, while the mean proportions of viable seeds for the two medicinal plants reached 91% and 87%, respectively. Seeds of Asclepias curassavica and Amaranthus exhibited certain tolerance to drought and salt stress during germination and strong tolerance to acid stress. The two plants could employ germination strategies of advancing, delaying, or entering dormancy to adapt to different stress environments. This study elucidated the germination characteristics and differences of Asclepias curassavica and Amaranthus seeds under three abiotic

stress factors, providing a theoretical foundation for the production and quality improvement of these two medicinal plants.

Full Text

Effects of Drought, Salt, and Acid Stress on Seed Germination of Medicinal Plants *Asclepias curassavica* and *Amaranthus tricolor*

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Abstract

This study investigated the response of two medicinal plants, *Asclepias curassavica* and *Amaranthus tricolor*, to environmental stresses using polyethylene glycol (PEG-6000), sodium chloride (NaCl), and pH gradient solutions to simulate drought, salt, and acid stress conditions. The results provide a theoretical basis for the introduction, cultivation, and production of high-quality medicinal materials in arid and saline-alkali regions. The findings revealed that both PEG and NaCl treatments significantly inhibited seed germination in both species, with germination percentage and germination index decreasing as concentrations increased. When subjected to 20% PEG, germination was severely suppressed, with rates below 50.00% and nearly complete inhibition at 25.00% PEG. After 14 days of stress, non-germinated seeds that were rehydrated showed rapid compensatory germination, indicating retained viability. TTC staining of rehydrated, non-germinated seeds confirmed that seeds remained viable, with viable seed proportions significantly higher than or not significantly different from the control, averaging 84% and 90% for *A. curassavica* and *A. tricolor*, respectively. Under pH 3.5–4.5, both species maintained germination rates exceeding 50%, with non-germinated seeds showing recovery upon rehydration and TTC staining results indicating high viability. However, approximately 10% of *A. curassavica* seeds and 15% of *A. tricolor* seeds lost viability under acid stress, though viable seed proportions remained high at 91% and 87%, respectively. Both medicinal plants demonstrated tolerance to drought and salt stress during germination and strong tolerance to acid stress. The two species can adopt different germination strategies—including advanced, delayed, or dormant germination—to adapt to various stress environments. This study clarifies the germination characteristics and differences of *A. curassavica* and *A. tricolor* seeds under three abiotic stress factors, providing a theoretical foundation for the production and quality improvement of these medicinal plants.

Keywords: medicinal plant; *Asclepias curassavica*; *Amaranthus tricolor*; seed germination; tolerance

Introduction

Global climate change and unsustainable land use practices have led to declining soil quality and productivity, including acidification and salinization, which severely restrict seed germination and growth of medicinal plants, thereby affecting their quality and yield. These abiotic stress factors have become primary constraints to the sustainable development of China' s traditional Chinese medicine industry. High concentrations of salt solution induce physiological drought in plants, disrupt intracellular ion balance, and reduce enzyme activity. As soil pH decreases, base cations such as potassium and phosphorus are leached, and soluble aluminum increases, leading to significantly reduced root growth, hindered nutrient absorption, and decreased resistance to pathogens. Drought stress generates various reactive oxygen species that are toxic to cells, disrupting normal growth and even causing plant death.

Asclepias curassavica (Asclepiadaceae) is a perennial herb named after the god of medicine. With bitter and cold properties, it is used to treat conditions including asthma, diarrhea, and rheumatism, and has hemostatic, anti-inflammatory, and wound-healing effects. *Amaranthus tricolor* (Amaranthaceae) is an annual plant with multiple uses as a vegetable, grain, and medicine, earning it the names "vitamin pill among vegetables" and "blood-enriching vegetable." Both species are cultivated primarily in provinces south of the Yangtze River in China and reproduce through seeds. Current research on these plants has focused on tissue culture and other aspects, but studies on their seed germination responses to drought are scarce, particularly in the acidic soils widely distributed in southern China. Wang et al. found that salt stress inhibited *A. tricolor* seed germination but did not determine whether non-germinated seeds remained viable under salt stress.

Seed germination represents a critical stage in the plant life cycle and a key phase for seedling establishment and population renewal, providing important guidance for medicinal plant applications. While water, soil pH, and salinity can act as stress factors during germination, environmental stress often plays an indispensable role in forming the superior characteristics of genuine medicinal materials. This study systematically investigated the germination characteristics of *A. curassavica* and *A. tricolor* seeds in response to environmental stress using PEG-6000, NaCl, and pH gradient solutions to simulate drought, salt, and acid conditions. The objective was to provide a theoretical basis for introducing and cultivating these species in arid and saline-alkali regions and for producing high-quality medicinal materials.

Materials and Methods

1.1 Experimental Materials

Seeds of *A. curassavica* were collected in October 2023 from the Medicinal Plant Garden at Guangxi Eco Engineering Vocational and Technical College. Seeds of *A. tricolor* were collected in October 2023 from areas surrounding the college campus (24°16 N, 109°13 E, altitude 108 m). Collected seeds were air-dried, cleaned, and stored at 4°C.

1.2 Experimental Design

Following the method of Michel and Kaufmann (1973), drought stress was simulated using PEG-6000 solutions at mass fractions of 5%, 10%, 15%, 20%, and 25%. Salt stress was simulated using NaCl solutions at concentrations of 50, 100, 150, and 200 $mmol \cdot L^{-1}$. Acid stress was simulated using CH_3COOH solutions at pH levels of 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0, with distilled water treatment as the control (CK). The experiment comprised 17 treatment groups. The paper culture method was used for germination tests, with three replicates per treatment. Culture conditions were maintained at 25°C with a 12 h light/12 h dark photoperiod.

1.3 Seed Germination Method

Seeds were placed in petri dishes lined with two layers of filter paper, with 50 seeds per dish. Ten milliliters of the respective treatment solution was added. The germination period under each stress treatment was 14 days. To determine the viability of non-germinated seeds after stress treatment, intact non-germinated seeds were counted on day 14 and transferred to distilled water for rehydration germination for an additional 7 days. Seeds that did not germinate after rehydration were subjected to TTC (2,3,5-triphenyltetrazolium chloride) staining to assess viability; embryos stained red indicated viable seeds. All germination experiments considered seeds germinated when the radicle emerged from the seed coat, with germinated seeds counted daily.

1.4 Data Processing

Germination percentage, germination index, and other metrics were calculated using the following formulas:

- **Germination percentage** = (Number of germinated seeds / Total number of seeds tested) × 100%
- **Germination index** = Σ(Gt / Dt), where Gt is the number of germinated seeds on day t and Dt is the corresponding germination day
- **Recovery germination percentage** = (Number of germinated seeds after rehydration / Total number of seeds tested) × 100%
- **Viable seed proportion** = (Recovery germination percentage + Number of stained seeds / Total number of seeds tested) × 100%

Data were analyzed using SPSS 19.0 software. One-way ANOVA was performed on germination percentage and germination index, with Duncan's multiple range test used to determine significant differences among treatments. Figures were prepared using Excel 2009.

Results

2.1 Effects of Different Stress Treatments on Germination Indices

PEG concentration significantly affected seed germination of both species in a similar manner. As PEG concentration increased, germination percentage and germination index decreased significantly compared to the control. At 15% PEG, germination of both species was significantly inhibited, with rates below 50.00%; at 20% PEG, germination was almost completely inhibited, with fewer than 5.00% of seeds germinating.

Salt concentration also significantly impacted germination. All salt treatments resulted in significantly lower germination percentages and indices compared to the control, with a significant declining trend as NaCl concentration increased. At 50 $\text{mmol} \cdot \text{L}^{-1}$, both species showed maximum germination metrics among salt treatments, but germination was still significantly inhibited, with rates and indices maintained at only 6.92% and 33.17% for *A. curassavica* and *A. tricolor*, respectively. No seeds germinated at concentrations $ 100*mmol* · *L*^{-1}$.

Acid stress produced different germination patterns between the two species. Acid stress inhibited *A. curassavica* germination, with the highest germination percentage and index (72.53% and 14.17%, respectively) observed at pH 3.5, though these values were 74.25% lower than the control and significantly different. Germination metrics showed irregular trends with increasing pH. In contrast, acid stress promoted *A. tricolor* germination, with over 78.13% of seeds germinating at pH 3.5–4.5, higher than the control. The number of germinated seeds decreased sharply at pH $ $5.0, though the germination index showed no significant difference from the control. These results indicate that acid stress, to some extent, benefited germination of both species [Figure 1: see original paper].

2.2 Effects of Different Stress Treatments on Germination Process

Under PEG stress, the germination process of both species was similar to the control, though PEG concentration primarily affected initial germination time and the number of seeds germinating during the peak period. For *A. curassavica*, control seeds began germinating on day 2, with peak germination occurring on day 4, during which 88.43% of total germinated seeds emerged. Under 5% and 10% PEG, initial germination time remained the same as the control, but the proportion of seeds germinating during the peak period decreased from 88.43% to 81.87% and 78.13%, respectively. As PEG concentration increased, initial germination was delayed to day 3 at 15% PEG and to day 4 at 20% PEG, with the proportion of seeds germinating during the peak period rapidly decreasing to 31.96% and 19.79%, respectively.

For *A. tricolor*, control seeds also began germinating on day 2, with peak germination on day 3, during which 78.13% of total germinated seeds emerged. Except for the 20% PEG treatment, which showed no germination throughout the experiment, other PEG concentrations yielded similar germination processes to the control, with peak-period germination accounting for 82.09%, 88.43%, and 78.97% of total germination under 5%, 10%, and 15% PEG, respectively.

NaCl stress had similar effects on germination process for both species. For *A. curassavica*, control seeds began germinating on day 2, with initial germination delayed to day 3 at 50 $mmol \cdot L^{-1}$ and fewer than 2.00% of seeds germinating. No germination occurred at higher concentrations. For *A. tricolor*, initial germination occurred on day 2 at 50 $mmol \cdot L^{-1}$, but the number of germinated seeds decreased by nearly 30.00% compared to the control. No seeds germinated at concentrations $ 100mmol · L^{-1}$.

Acid stress affected germination process differently. For *A. curassavica*, initial germination time was consistent with the control at pH 3.5–4.5, but the proportion of seeds germinating during the peak period decreased from 88.43% to 72.53% and 74.26%, respectively. For *A. tricolor*, acid stress promoted earlier germination, with initial germination advanced to day 1 at pH 3.5–4.5, and the proportion of seeds germinating during the peak period increasing from 78.13% to 88.25% and 82.00%, respectively [Figure 2: see original paper].

2.3 Effects of Different Stress Treatments on Seed Viability

The fate of non-germinated seeds under stress includes three possibilities: viable seeds that can germinate after rehydration, viable seeds that remain dormant after rehydration, or non-viable seeds that have lost vitality.

After PEG stress was relieved through rehydration, intact seeds of both species germinated in large numbers. Recovery germination rates increased with original PEG concentration. Even for seeds with 0.00% germination at 20% PEG (*A. curassavica*) and 5.84% germination at 20% PEG (*A. tricolor*), recovery germination rates reached 71.25% and 78.97%, respectively. TTC staining showed that the proportion of stained seeds decreased with increasing original PEG concentration. Both species maintained high viable seed proportions, with means of 88.43% and 91.32% for *A. curassavica* and *A. tricolor*, respectively, indicating that both medicinal plant seeds can employ delayed germination and dormancy strategies to cope with drought .

NaCl stress produced contrasting recovery and dormancy patterns between the two species. After stress relief, *A. curassavica* showed recovery germination rates of 11.60%–36.28% that decreased with increasing original NaCl concentration, while 46.54%–71.67% of seeds remained dormant, with dormancy rates increasing with NaCl concentration. In contrast, *A. tricolor* showed recovery germination rates exceeding 64.73% that increased with NaCl concentration, with fewer than 7.01% of seeds remaining dormant. TTC staining indicated that both species maintained high viable seed proportions (71.19%–94.04%), demonstrating different adaptive responses to salt stress. However, high NaCl concentrations caused approximately 10% seed death in both species .

Acid stress also showed contrasting effects. After stress relief, non-germinated *A. curassavica* seeds showed recovery germination rates of only 18.18%-31.15%, with 7%-12% remaining dormant, while approximately 18%-31% of seeds died due to stress damage. Non-germinated *A. tricolor* seeds showed maximum recovery germination of only 1.52%, with virtually no dormancy and almost all non-germinated seeds dying from stress damage. However, viable seed proportions under acid stress were not significantly different from or were significantly higher than the control (83.45%-92.92%), indicating that acid stress did not significantly reduce seed vitality in either species, though it caused approximately 10–15% seed death .

Discussion

Seed germination is a sensitive stage in plant responses to environmental stress. Water is the primary factor affecting seed germination and early seedling establishment. PEG treatment is widely used to study drought tolerance during germination. This study found that *A. curassavica* and *A. tricolor* seeds were sensitive to drought stress, with germination significantly inhibited at 15% PEG, consistent with results for *Rhododendron latoucheae* seeds. As drought stress increased, germination was almost completely inhibited, similar to the pattern observed in *Sorghum halepense* seeds. However, after stress relief, large numbers of intact seeds from both species germinated upon rehydration, with recovery germination rates increasing with original PEG concentration. This indicates that short-term water stress affects germination but does not cause loss of viability, and may even promote germination after stress relief, demonstrating that both medicinal plants possess drought tolerance.

Soil salinity affects seed germination, with lower concentrations often promoting and higher concentrations inhibiting germination—a "low-promotion, high-inhibition" effect. However, this pattern varies by species. This study found that salt stress inhibited germination in both species, with inhibition increasing with NaCl concentration, consistent with the response of *Lepidium latifolium*. When salt stress was relieved, *A. curassavica* seeds entered a dormant state to maintain viability, while *A. tricolor* seeds showed rapid and extensive germination, indicating high recovery capacity. These different adaptive responses show that both species are sensitive to salt during germination, making salt a limiting factor. For cultivation in saline-alkali soils, fields with lower salinity should be selected or water management should be optimized before planting to improve germination rates.

Soil pH often becomes a limiting factor for seed germination. Most plant seeds show inhibited germination in excessively high or low pH environments, with reduced germination percentages, delayed initial germination, and poor seedling uniformity. However, some species show broad pH tolerance. This study demonstrated that both species have strong acid tolerance, with over 57% of *A. curassavica* seeds and 83% of *A. tricolor* seeds germinating at pH 3.5–4.5. Acid stress not only promoted *A. tricolor* germination but also accelerated it, consistent with previous findings. Both species are suitable for planting in increasingly acidified soils in southern China.

Considering germinated seeds during stress, recovered seeds after rehydration, and dormant seeds as all viable, the proportion of viable seeds reflects the degree of adaptation to stress and the germination strategies employed. The results show that both species can sensitively detect changes in water and salt availability. Under mild stress, they germinate slowly with few seeds; as stress increases, large numbers of seeds enter dormancy to maintain long-term viability for germination when conditions become favorable. Although PEG and NaCl treatments inhibited germination, both species maintained high viable seed proportions that could form transient seed banks in natural environments. Additionally, small numbers of viable seeds remained ungerminated even after stress and rehydration, potentially forming persistent seed banks for population maintenance. This reflects that *A. curassavica* and *A. tricolor* can employ multiple germination strategies to distribute unpredictable environmental risks, providing sources and momentum for future population recruitment.

In summary, *A. curassavica* and *A. tricolor* seeds exhibit tolerance to drought and salt stress and strong tolerance to acid stress during germination, employing different strategies to adapt to various stress environments. In practice, mild drought or salt stress can be applied to promote germination, but high-salinity environments should be avoided or soil salinity should be regulated. Acidic soil environments cause minimal inhibition of *A. curassavica* germination while promoting and accelerating *A. tricolor* germination, demonstrating strong adaptability to acidic conditions.

Conclusions

1. PEG treatment significantly inhibited seed germination in both *A. curassavica* and *A. tricolor*, with germination percentage and index decreasing as PEG concentration increased. Acid stress significantly inhibited *A. curassavica* germination but promoted *A. tricolor* germination, with increased germination percentages and indices.

2. PEG stress delayed germination speed in both species to varying degrees. Specifically, 15% PEG delayed initial germination to day 3 for *A. curassavica* and day 3 for *A. tricolor*, while 20% PEG delayed initial germination to day 4 for *A. curassavica*. Under acid stress at pH 3.5–4.5, initial germination time for *A. curassavica* was similar to the control, while *A. tricolor* germination was accelerated by 1–2 days.

3. After rehydration of non-germinated seeds from PEG treatments, large numbers of seeds from both species germinated, indicating retained viability. Various NaCl concentrations caused approximately 10% seed death in both medicinal plants.

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