Postprint: Effects of Salt Stress on Quinoa Seed Germination

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Abstract

Reclaimed water can be used to irrigate farmland, but the anions contained in the water can cause salt stress in soil. To investigate the effects of salt stress on seed germination characteristics and radicle and plumule growth of quinoa (Chenopodium quinoa), this study used six quinoa varieties (Red, Guohong, Taihong, Taizihong, Yellow, Taihuanghong) as materials. Seeds of the six quinoa varieties were treated with NaCl, Na2SO4, NaHCO3, and control (CK), respectively. Indices such as germination rate, radicle and plumule inhibition rates were measured. The mean-square deviation decision method was used to comprehensively evaluate the salt tolerance of different quinoa varieties, and preliminarily screen varieties with stronger salt tolerance under different salt stresses. The results showed that among the three salt stresses, Na2SO4 had the most significant inhibitory effect on seed germination indices. The germination rates of all six quinoa varieties were relatively low, remaining below 5%. Except for Yellow quinoa and Taihuanghong quinoa, the vigor index and growth rate of the other four varieties were both 0. Except for Yellow quinoa, the radicle and plumule inhibition rates of the other five quinoa varieties all reached 100% under Na2SO4 treatment. NaCl had a smaller inhibitory effect on seed germination and growth, and could even promote radicle and plumule growth. The growth rates of Guohong quinoa and Taihuanghong quinoa under NaCl treatment were consistently higher than the control. At 9 h and 21 h, the radicle inhibition rates of Guohong quinoa were -28.32% and -37.57%, respectively. Using the mean-square deviation decision method to comprehensively evaluate the germination and growth indices of the six quinoa varieties, Guohong quinoa showed higher resistance to NaCl and NaHCO3, while Yellow quinoa showed higher resistance to Na2SO4. Based on these results, salt stress is detrimental to quinoa seed germination and growth. However, planting suitable varieties in different saline regions can improve quinoa survival rates and growth quality, thereby meeting the requirements for quinoa landscaping and reclaimed water irrigation.

Full Text

Preamble

Effects of Salt Stress on Quinoa Seed Germination

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Abstract

Reclaimed water can be used for farmland irrigation, but the anions it contains can cause soil salt stress. To investigate the effects of salt stress on seed germination characteristics and radicle and germ growth of quinoa (*Chenopodium quinoa*), this study examined six quinoa varieties (Red, China Red, Tai Red, Tai Purple-Red, Yellow, and Tai Yellow-Red) under NaCl, Na_2SO_4, $NaHCO_3$, and control (CK) treatments. Germination rate, radicle and germ inhibition rates, and other indices were measured, and the mean square error decision method was used to comprehensively evaluate the salt tolerance of different quinoa varieties to preliminarily screen for varieties with stronger salt tolerance under different salt stresses. The results showed that among the three salt treatments, Na_2SO_4 had the most pronounced inhibitory effect on seed germination indices. All six quinoa varieties exhibited relatively low germination rates, consistently remaining below 5%. Except for Yellow and Tai Yellow-Red quinoa, the other four varieties showed zero vigor index and growth rate. Except for Yellow quinoa, Na_2SO_4 achieved 100% inhibition rates for radicle and germ growth in the remaining five varieties. NaCl showed relatively minor inhibition on seed germination and growth, and even promoted radicle and germ growth. The growth rates of China Red and Tai Yellow-Red quinoa under NaCl treatment remained higher than the control throughout the experiment, with radicle inhibition rates of -28.32% and -37.57% at 9 h and 21 h, respectively. Using the mean square error decision method to comprehensively evaluate the germination and growth indices of the six quinoa varieties, China Red quinoa showed higher resistance to NaCl and $NaHCO_3$, while Yellow quinoa showed higher resistance to Na_2SO_4. Overall, salt stress adversely affected quinoa seed germination and growth; however, planting appropriate varieties in different saline regions can improve quinoa survival rates and growth quality to meet the requirements for landscaping and reclaimed water irrigation.

Keywords: Quinoa, salt stress, seed germination, germination vigor

Introduction

China faces severe water shortages with scarce per capita water resources, creating numerous contradictions between urban water supply and demand (Bai Baoxun, 2010). Utilizing reclaimed water as an irrigation source has become a key approach to address water scarcity and pollution problems. Reclaimed water—treated urban wastewater containing nitrogen, phosphorus, organic matter, and other nutrients—can improve soil conditions and provide nutritional elements for plants (Hu Tingfei et al., 2020). However, studies indicate that reclaimed water contains substantial $HCO_3{}^-$, Na^+, Mg^{2+}, and Ca^{2+} ions (Zhao Quanyong et al., 2017), which increase soil salinity after irrigation, causing soil salinization and salt damage stress to plants. Under salt stress, plants primarily regulate osmotic pressure by absorbing inorganic salt ions to prevent cellular dehydration (Yang Xiaoju et al., 2013). However, high-salt environments cause excessive Na^+ influx into cells, disrupting ionic homeostasis and creating both osmotic and ionic stress that alters intracellular ion concentrations and composition (Ding Junnan and Chi Defu, 2014). This subsequently triggers changes in plant physiology (Zhu Jinfang et al., 2015), endogenous hormones (Zhang Min et al., 2008), enzyme systems (Sun Guorong et al., 2001), and morphology (Yu Chang et al., 2014), potentially causing plant wilting and death under severe conditions. Therefore, understanding plant adaptability to saline-alkaline environments and screening salt-tolerant plants are crucial for reclaimed water utilization and urban water conservation.

Quinoa (*Chenopodium quinoa*), an annual plant in the Amaranthaceae family, produces seeds rich in protein, vitamins, amino acids, minerals (Bhargava et al., 2007; Antonio et al., 2010), and lysine—an essential amino acid lacking in other plants (Wang Liming et al., 2014). Consequently, quinoa holds broad development prospects in the food industry. Beyond its nutritional value, quinoa offers high ornamental value due to its colorful inflorescences (white, yellow, deep red, purple, etc.). If these visually appealing quinoa cultivars could be incorporated into urban landscaping, they would diversify greening patterns and create unique crop-based landscapes in cities. Quinoa also possesses certain salt-alkaline tolerance, though tolerance thresholds vary among varieties, and excessively high salt concentrations can still cause plant death. Gu Minfeng et al. (2017) found that 0.6% NaCl concentration promoted growth of Yanli 47 and 48 quinoa seeds, while 1.8% NaCl concentration resulted in nearly 100% root inhibition rates, severely suppressing seed growth. Yang Farong et al. (2017) studied physiological responses of three quinoa varieties under NaCl stress and found that Longli No. 1 exhibited significantly higher soluble sugar, soluble protein, and proline contents at $500\ \mathrm{mmol \cdot L^{-1}}$ concentration than the other two varieties, indicating good salt tolerance. Therefore, this study investigated germination characteristics of different quinoa varieties under salt stress and their responses to various salt types, using the mean square error decision method to comprehensively evaluate germination indices and screen for quinoa varieties with multiple salt tolerance traits, aiming to provide a theoretical basis for salt tolerance screening and landscaping applications.

Materials and Methods

1.1 Experimental Materials

Six quinoa varieties (Red, China Red, Tai Red, Tai Purple-Red, Yellow, and Tai Yellow-Red) were provided by Inner Mongolia Yiji Company.

1.2 Experimental Design

A filter paper petri dish germination test was conducted. Petri dishes were cleaned with distilled water, sterilized in an autoclave, and dried. Uniform, plump, pest-free quinoa seeds were selected and disinfected with 0.5% potassium permanganate for 30 minutes, then rinsed with distilled water. One hundred seeds were placed in each petri dish pre-lined with two layers of filter paper, and 5 mL of 200 $\mathrm{mmol \cdot L^{-1}}$ NaCl, Na_2SO_4, or $NaHCO_3$ solution was added. A distilled water treatment served as the control (CK), with four replicates per treatment. The petri dishes were incubated at 25°C, and seed germination counts were recorded every 3 hours starting at 6 h. Radicle and germ lengths were measured concurrently.

1.3 Seed Germination Index Calculation

Seed germination indices were calculated using the following formulas (Huang Zhenying et al., 2001; Mao Peichun and Wang Yong, 2004; Gao Shaomin et al., 2019):

Germination rate: $G = n/N \times 100\%$
where n is the number of germinated seeds and N is the total number of seeds in the petri dish.

Germination index: $Gi = \sum(Gt/Dt)$
where Gt is the number of germinated seeds on day t and Dt is the corresponding germination day.

Vigor index: $Iv = S \times Gi$

Growth rate: $L/\sum[Ni \times (Dt - Di + 0.5)]$

Radicle elongation inhibition rate: (Control radicle length−Treatment radicle length)/Control radicle length× 100%

Germ elongation inhibition rate: (Control germ length−Treatment germ length)/Control germ length× 100%

where L is the total radicle length of all germinated seeds in each petri dish; S is the sum of radicle length and hypocotyl length (in cm); Ni is the number of germinated seeds on day i; Dt is the total experimental duration in days; and Di is day i.

1.4.1 Evaluation Index Standardization

The range transformation method (Yi Pengtao et al., 2018) was used for dimensionless standardization of data. After standardization, all indices fall within the range [0,1] and are converted to positive indicators, where optimized results approach 1 and poorest results approach 0. The calculation formulas are as follows:

For positive indicators: $G_{ij} = \frac{B_i - B_{\min}}{B_{\max} - B_{\min}}$

For negative indicators: $G_{ij} = \frac{B_{\max} - B_i}{B_{\max} - B_{\min}}$

where G_{ij} is the standardized value of the indicator; B_i is the measured value of the ith indicator; $B_{\max}$ is the maximum value of the indicator; and $B_{\min}$ is the minimum value.

1.4.2 Comprehensive Evaluation Based on Mean Square Error Decision Method

The mean square error decision method was used to comprehensively evaluate germination indices of six quinoa varieties under three salt stresses. Standardized values served as random variable values for each evaluation indicator to calculate means and mean square errors, which were then normalized to obtain weight coefficients for each indicator. Finally, weight coefficients and standardized germination indices were used to calculate comprehensive scores for six quinoa varieties under different salt stresses (Yi Pengtao et al., 2018). The specific calculation steps are:

(1) Calculate the mean $E(G_j)$ of random variables: $E(G_j) = \frac{1}{n}\sum_{i=1}^{n} G_{ij}$

(2) Calculate the mean square error of indicator set G_j: $\sigma(G_j) = \sqrt{\sum_{i=1}^{n}[G_{ij} - E(G_j)]^2}$

(3) Calculate the weight coefficient of indicator set G_j: $W_j = \frac{\sigma(G_j)}{\sum_{j=1}^{m}\sigma(G_j)}$

(4) Multi-indicator decision-making and ranking: $D_i(W) = \sum_{j=1}^{m} W_j \times G_{ij}$

1.4.3 Single-Factor Analysis of Variance

Data were organized using Microsoft Excel 2018. SPSS Statistics 20 was used for one-way ANOVA analysis, with LSD and Duncan' s multiple comparison tests for significance testing ($P < 0.05$). Origin 2018 was used for graphing.

Results

2.1 Effects of Three Salt Stresses on Quinoa Germination Rate

Germination rates of salt-treated quinoa were generally higher than the control after 18 h until the experiment ended [Figure 1: see original paper]. Different salt stresses showed varying inhibition effects on seed germination rates. Na_2SO_4 exhibited the most obvious inhibition on all six quinoa varieties, with germination rates fluctuating slightly over time and remaining relatively low, consistently below 5%. The highest germination rate occurred in China Red quinoa at 30 h, reaching 4.75% (Figure 1B). Under $NaHCO_3$ treatment, Red, Tai Red, Tai Purple-Red, and Tai Yellow-Red quinoa showed significant improvement in germination rates during the middle germination period (Figure 1A, C, D, E). The highest germination rate was observed in Tai Purple-Red quinoa at 18 h, reaching 14.5%, which was 3.87 and 6.44 times higher than NaCl and Na_2SO_4 treatments at the same time point.

2.2 Effects of Three Salt Stresses on Quinoa Germination Index

All six quinoa varieties treated with Na_2SO_4 showed the lowest germination indices [Figure 2: see original paper]. The lowest value occurred in Tai Red quinoa at 33 h (3.83), which was 51.03% and 75.23% lower than NaCl and $NaHCO_3$ treatments, respectively (Figure 2C). $NaHCO_3$-treated quinoa showed the highest germination indices (excluding the control), with Yellow quinoa reaching a maximum of 16.2 at 33 h, which was 13.45% and 34.70% higher than NaCl and Na_2SO_4 treatments, respectively (Figure 2E). Among the six varieties, Tai Red, Tai Purple-Red, and Tai Yellow-Red quinoa showed higher germination indices under Na_2SO_4 treatment than under $NaHCO_3$ during the first 12 h, but after 12 h, $NaHCO_3$ treatment produced higher indices than Na_2SO_4 (Figure 2C, D, F). China Red quinoa showed this transition at 18 h (Figure 2B).

2.3 Effects of Three Salt Stresses on Quinoa Vigor Index

The vigor index represents the germination potential, growth capacity, and production potential of seeds under rapid and uniform germination. Under adverse conditions, higher vigor indices indicate better stress resistance (Wu Yan et al., 2004). NaCl showed lower inhibition on quinoa compared to the other two salts. Among the six varieties, China Red quinoa demonstrated higher resistance to NaCl, with its vigor index significantly higher than the other five varieties. At 33 h, China Red quinoa showed vigor indices 47.66% (Figure 3A), 73.31% (Figure 3C), 63.85% (Figure 3D), 59.90% (Figure 3E), and 75.68% (Figure 3F) higher than Red, Tai Red, Tai Purple-Red, Yellow, and Tai Yellow-Red quinoa, respectively. Red, China Red, Tai Red, and Tai Purple-Red quinoa showed minimal resistance to Na_2SO_4, with vigor indices of zero (Figure 3A, B, C, D).

2.4 Effects of Three Salt Stresses on Quinoa Radicle Growth Rate

The three salt solutions differentially affected quinoa radicle growth rates. Initially, NaCl-treated Red quinoa showed higher growth rates than the control, but over time, these rates continuously declined, falling below the control at 12 h and reaching a minimum of 0.14 at 30 h (Figure 1A). NaCl-treated China Red quinoa exhibited radicle growth rates higher than the control, peaking at 5.71 times the control value at 9 h, while Na_2SO_4 treatment resulted in zero growth rate (Figure 1B). $NaHCO_3$-treated Tai Purple-Red quinoa showed a growth rate of 0.78 at 6 h, which was 2.05 and 9.15 times higher than the control and Na_2SO_4 treatments, respectively, but then sharply declined to near zero by 33 h [Figure 4: see original paper] (Figure 4D). NaCl had minimal impact on radicle growth rates and even promoted growth in China Red and Tai Yellow-Red quinoa (Figure 4B, F). However, Na_2SO_4 inhibited radicle growth, resulting in zero growth rates for four varieties (Red, China Red, Tai Red, and Tai Purple-Red) except Yellow and Tai Yellow-Red quinoa (Figure 4A, B, C, D).

2.5 Effects of Three Salt Stresses on Quinoa Radicle Inhibition Rate

Over time, NaCl showed increasing radicle inhibition rates on Red quinoa, rising from 12.47% initially to 85.02% at 30 h, while $NaHCO_3$ inhibition increased from 84.85% to 98.24% [Figure 5: see original paper] (Figure 5A). NaCl maintained relatively low inhibition on China Red quinoa, with the inhibitory effect weakening over time. The inhibition rate was 42.92% at 6 h and 12.11% at 30 h, with negative values of -28.32% and -37.57% at 9 h and 21 h, respectively, indicating that NaCl promoted radicle growth (Figure 5B). Salt solutions also affected the other four quinoa varieties, with inhibition rates increasing over time. Na_2SO_4 showed the strongest radicle inhibition, achieving 100% inhibition rates in five varieties except Yellow and Tai Yellow-Red quinoa (Figure 5A, B, C, D). The order of radicle inhibition effects was: $Na_2SO_4 > NaHCO_3 >$ NaCl.

2.6 Effects of Three Salt Stresses on Quinoa Germ Inhibition Rate

At 6 h of salt stress, all three salts achieved 100% inhibition rates on China Red quinoa [Figure 6: see original paper] (Figure 6B), while showing 0% inhibition on the other five varieties (Figure 6A, C, D, E, F). Starting from 9 h, salt solutions began inhibiting germ elongation, with Na_2SO_4 maintaining 100% inhibition rates (Figure 6A, B, C, D, E). NaCl-treated China Red quinoa showed inhibition rates below 0% from 15 h, reaching a minimum of -81.25% at 21 h (Figure 6B). Na_2SO_4-treated Tai Yellow-Red quinoa showed germ elongation inhibition rates below 100%, decreasing over time from 97.47% at 6 h to 77.49% at 33 h—a reduction of 20.50%—demonstrating relatively high salt stress resistance (Figure 6F).

2.7 Comprehensive Evaluation of Quinoa Seed Germination

The mean square error decision method was applied to comprehensively evaluate germination indices under three salt treatments, with scores and rankings shown in Tables -. This evaluation effectively identified the most salt-tolerant quinoa varieties by integrating multiple germination indicators.

The mean square error decision method revealed that under NaCl treatment, China Red quinoa showed the best germination performance, while Tai Red quinoa performed worst. Under Na_2SO_4 treatment, Yellow quinoa performed best, while Tai Red quinoa performed worst. Under $NaHCO_3$ treatment, China Red quinoa performed best, while Tai Red quinoa performed worst.

Discussion

Seed germination marks the beginning of plant life and represents the most critical yet vulnerable stage of plant development, being highly susceptible to environmental influences, particularly saline-alkaline stress (Li Yaping et al., 2019). Evaluating plant salt tolerance during germination is a common breeding approach. Germination rate, germination index, vigor index, and growth rate are commonly used indicators to measure seed germination and comprehensively reflect salt stress effects (Yang Yongyi et al., 2019; Chen Yaxin et al., 2019). In this study, six quinoa varieties showed relatively consistent patterns in germination rate, germination index, vigor index, and growth rate under three salt influences: germination rate and growth rate initially increased then decreased, while germination index and vigor index increased slowly over time. This suggests that salt stress had relatively minor effects on the quinoa seed germination stage. During germination, quinoa initiated osmotic regulation in response to salt stress "stimulation," accelerating seed growth rates to ensure normal germination. However, prolonged stress led to excessive ion accumulation, creating ionic stress. The combined effects of osmotic and ionic stress ultimately prevented normal germination, causing germination rates to decline. Similar effects of salt stress on oat (Luo Zhina et al., 2012) and Siberian elm seed germination (Zhu Jianfeng et al., 2020) have been reported. Salt stress affects seeds at germination, morphological, cellular, physiological, and molecular levels, severely inhibiting plant growth under intense stress (Li Yaping et al., 2019; Chen Yaxin et al., 2019). Zhao Ying et al. (2019) studied mixed saline-alkaline stress effects on quinoa seed germination and found that neutral salts NaCl and Na_2SO_4 had relatively low inhibition. However, this study found that Na_2SO_4 and alkaline salt $NaHCO_3$ strongly inhibited radicle and germ growth, with Na_2SO_4 achieving 100% inhibition rates in five varieties except Yellow quinoa. NaCl showed the lowest inhibition degree and even promoted radicle and germ growth. These findings align with Pan Pingxin (2021) and Wang Xiaohang (2020), while Li Zhen (2019) and Wei Hongping (2020) reported NaCl inhibition of plant growth. The inhibitory effects of NaCl stress vary across plant species because different plants have different salt tolerance capacities and physiological metabolic differences (Li Shanjia et al., 2016), which also applies to different varieties. Alkaline and salt stresses have different inhibition mechanisms: salt stress primarily inhibits plant growth through osmotic effects and ion toxicity, while alkaline stress additionally includes high pH effects that may cause ionic imbalance and increase damage (Zhang Tongying et al., 2019). This aligns with many domestic studies on plant saline-alkaline tolerance: alkaline stress caused greater damage than salt stress to *Leymus chinensis* and *Helianthus annuus* (Yan Hong et al., 2005); low-concentration neutral salts promoted cork oak seed germination while alkaline salts inhibited it (Li Zhiping et al., 2015); similar effects were observed on kidney bean (*Phaseolus vulgaris*) seed germination (Zheng Lina et al., 2018). Reclaimed water still contains certain ions after treatment that inhibit plant growth, so reclaimed water irrigation should prioritize salt-tolerant plants. This study found that salt stress inhibited quinoa seed germination and growth, with neutral salt NaCl having the smallest impact and even promoting growth in some varieties, while alkaline salts completely inhibited quinoa growth. Therefore, when using reclaimed water for irrigation in quinoa cultivation areas, alkaline ions should be removed as much as possible to ensure normal plant growth.

Plant salt tolerance is a complex comprehensive trait resulting from interactions among multiple factors, and different salt tolerance indicators may yield different results (Li Zhen et al., 2019), necessitating comprehensive evaluation of all indicators. The membership function method is commonly used for comprehensive evaluation but suffers from poor objectivity and strong subjectivity. The mean square error decision method is frequently applied in urbanization level and intensity evaluations (Zhu Jing et al., 2020; Ren Caifeng et al., 2019) and has been used to evaluate grasslands under different cultivation measures by objectively assigning weights through indicator calculations (Dong Yunlong et al., 2015). This study objectively and comprehensively evaluated seed germination indices using the mean square error decision method, revealing that NaCl inhibited seed growth primarily by restricting radicle growth, with Tai Red, Red, and Yellow quinoa showing the highest radicle inhibition rates, while China Red quinoa showed the best germination performance with the fastest growth rate and least radicle and germ inhibition. Na_2SO_4 showed the most obvious inhibitory effect, primarily affecting vigor index, growth rate, radicle inhibition rate, and germ inhibition rate, with Yellow quinoa performing best among the six varieties in terms of germination rate, vigor index, and germination index. $NaHCO_3$ had greater effects on germ inhibition rate, with China Red quinoa performing best under this treatment, showing lower germination rate and germination index but higher vigor index and growth rate, along with lower radicle and germ inhibition rates than other varieties. Under salt stress, plant roots first perceive stress signals and generate corresponding physiological responses (Lu Yanmin, 2012). Zhang Lixia et al. (2015) studied saline-alkaline stress effects on prunella seed germination and found that young roots were more sensitive to salt stress. This study found that after normal germination, radicles directly contacted salt solutions, allowing more salt ions to enter the plant and further disrupting internal ion balance, ultimately causing death of normally germinated seeds. Tai Yellow-Red quinoa could germinate and grow under all three salt stresses and showed relatively good development under the harsh Na_2SO_4 environment, indicating strong adaptability to composite salt conditions and high potential for application. In summary, China Red quinoa is suitable for areas with severe NaCl and $NaHCO_3$ salinization; Yellow quinoa is suitable for areas with severe Na_2SO_4 salinization; and Tai Yellow-Red quinoa is suitable for areas with composite salinization.

Conclusion

Under three different salt stress treatments, significant differences were observed in germination rate, growth rate, vigor index, and root length of different quinoa varieties compared to the control. The comprehensive ranking of six quinoa varieties under NaCl treatment was: China Red > Red > Yellow > Tai Purple-Red > Tai Yellow-Red > Tai Red. Under Na_2SO_4 treatment: Yellow > Tai Yellow-Red > Red > China Red > Tai Purple-Red > Tai Red. Under $NaHCO_3$ treatment: China Red > Tai Yellow-Red > Yellow > Tai Purple-Red > Red > Tai Red. The overall inhibition degree of the three salt stresses on six quinoa varieties ranked as: Na_2SO_4 > $NaHCO_3$ > NaCl.

Based on these results, among the six quinoa varieties, China Red quinoa is suitable for areas with severe NaCl and $NaHCO_3$ salinization; Yellow quinoa is suitable for areas with severe Na_2SO_4 salinization; and Tai Yellow-Red quinoa is suitable for areas with composite salinization.

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Note: Figure translations are in progress. See original paper for figures.

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