Effect of Salt Stress on Seed Germination of Alfalfa

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Abstract

Soil salinity and Irrigation water salinityare one of the important factors affecting the germination process and survival rate of crop seeds. Determining the appropriate salt concentration range for seed germination is in order to seed germination and prevent the damage of emergence by excessive salt ions, and it is also the prerequisite for improving the utilization of seed resources in agricultural production.In order to reveal the germination process and physiological mechanism of salt tolerance of Medicago sativa Seeds under different salt stress conditions. In this experiment, we took Zhongmu No.3 as the research object, NaCl was used as the salt stress of seed germination and distilled water as the control (CK, NaCl concentration 0 mmol/L), and we set six different concentration of NaCl (25,50, 100, 150, 200, 250 mmol/L) , 4 repetitions for each processing set. Seeds were placed in a constant temperature light incubator in a Petri dish for seed germination test. The number of seeds germinated under each treatment was observed and recorded at a fixed time point every day. The germination rate, germination potential and germination index were calculated. The results showed that the germination rate of Zhongmu No.3 under 25 mmol/L salt concentration was same as the CK, they both were 99% and the germination potential was 4% lower than CK, but not significant. The germination rate and germination potential of alfalfa under other salt concentrations were significantly lower than CK. The germination rate of alfalfa under 250mmol/L salt concentration were 0.5% and the germination potential of alfalfa under 250mmol/L salt concentration were 0. The germination rate and germination potential of Zhongmu No.3 were negatively correlated with NaCl concentration (P<0.05). When NaCl concentration was higher than 25 mmol/L, the germination rate decreased by 4.5% with an increase of 10 mmol/L. With the increase of NaCl concentration, the germination peak of Zhongmu No.3 showed a lagging trend, and the seed germination delayed 6 days under 250mmol/L salt concentration. According to the regression analysis, the salt-tolerant half lethal concentration of Zhongmu No. 3 was 135 mmol/L, and the salt-tolerant lethal concentration was 223 mmol/L.The results showed that suitable salinity stress (25 mmol/L) could stimulate alfalfa seed germination and promote seedling growth. Higher salinity stress led to a significant linear decrease in seed germination rate. It is of great significance for planting alfalfa or irrigating alfalfa with brackish water in saline-alkali soil to ensure the soil salinity and irrigation water salinity in the appropriate range during alfalfa germination.

Keywords

ZhongmuNo.3, salt stress, germination rate, germination potential, salt tolerance threshold.

1. Introduction

Soil salinization is one of the major problems facing arid and semi-arid regions in China today. With global warming, reduced rainfall, and unreasonable irrigation factors, the problem of soil salinization in China has become increasingly serious, which has seriously affected food security and land resource development and utilization [1].Therefore, the scientific and rational development and utilization of salinized land and the development of suitable crops are of great strategic significance for improving the utilization of land resources and the sustainable development of agriculture.

Medicago sativa is the world's largest perennial herbaceous forage grass, known as the "king of pasture" [2]. It plays an important role in improving salinized soil, expanding effective arable land and improving the ecological environment. Plants are tolerant to saline-alkali environments at different stages of growth and are most sensitive during seed germination. At present, there have been many studies on abiotic stress and regulatory substances in the germination of alfalfa seeds, including the environment (high temperature and low temperature, flood and droughts, etc.), endogenous hormones (ABA, GA_3, etc.), and various foreign substances (Proline, sugar, vitamins, etc.) and saline-alkali species ($CaCl_2$, NaCl, Na_2SO_4, $NaHCO_3$, Na_2CO_3), etc. [3-6]. Sun Yuzhu [7] used different concentrations of NaCl to stress the alfalfa. With the increase of salt concentration, the germination time of alfalfa seeds was prolonged accordingly, and the germination rate index also decreased. When the salt concentration was 150 mmol/L, the salt pair Seed germination has a certain promoting effect, while high concentration of salt (300 mmol/L) inhibits seed germination. Jing Yanxia[8] showed that low concentration of sodium salt stress (NaCl, $NaHCO_3$, Na_2CO_3) had a certain inhibitory effect on seed germination; compared with neutral salt, alkaline ($NaHCO_3$, Na_2CO_3) harmed to alfalfa seeds the ability depends mainly on the pH value. The high pH value is more harmful to the germination rate and germination potential of the seed, while the Na^+ toxicity is second. The responses of different varieties of alfalfa to salinity were significantly different, and the planting area of alfalfa in Zhongmu 3 increased rapidly year by year. It is urgent to elucidate the response of seed germination under salt stress and the salt tolerance threshold suitable for germination. At present, there are still few research reports in this area. Therefore, this study investigated the effects of different NaCl concentration solutions on the germination process of alfalfa seeds, explored the tolerance mechanism and threshold range of salt concentration during seed germination, and provided scientific data support for determining the soil salinity suitable for the cultivation of Zhongmu No.3. Furthermore, it provides a scientific basis for improving the germination rate and survival rate of alfalfa seeds in the field, especially in saline-alkali or brackish water, and maximizing the development of forage planting by using saline-alkali land and brackish water resources.

2. Materials and Method

2.1 Experiment material

The experiment was carried out in the Jujube Central Laboratory of Hebei Agricultural University from September 15 to September 24, 2018. The test materials were Zhongmu No. 3 seeds with consistent color, uniform size, fullness and no damage to the seed coat. The main reagents, instruments and equipment required for the test include: NaCl (chemically pure), distilled water, sodium hypochlorite solution (chemically pure); constant temperature light incubator (LRH-250-G), electronic balance; beaker, volumetric flask, pipette, qualitative filter paper, petri dish, sealing film, etc.

2.2 Test design and method

NaCl was used as the stress factor for seed germination, and a total of 7 treatments were set. Using distilled water as control (CK, NaCl concentration 0 mmol/L), 6 different NaCl concentrations (T1-T6: 25, 50, 100, 150, 200, 250 mmol/L, respectively), 4 for each treatment repeat.

Before the test seeds were placed in the culture dish, they were sterilized with 0.5% sodium hypochlorite solution for 5 min, then thoroughly washed with distilled water, and finally dried with a filter paper. Place 2 layers of qualitative filter paper in a washing and disinfecting dish with a diameter of 9 cm and a height of 1.7 cm. Place 50 test seeds evenly on the filter paper, and pipette 5 ml of different concentrations of NaCl solution. The sealing film is then covered. In order to ensure the normal respiration during seed germination, 10 small holes (pore size of about 2 mm) are uniformly applied on the sealing film, and the sealing film is replaced when it is damaged.

The culture dishes were layered into a constant temperature light incubator, and the illumination and temperature were set to first light 16 h + temperature 25 ° C and then dark 8 h + temperature 18 ° C, so repeated alternating, the luminous flux was 150 μmol / m ^{2} s. After the start of the experiment, observe and record the number of germinated seeds and related parameters in each dish at 6:00 pm every day, and replace the solution in the dish (new solution volume is 5 ml). The germination standard is that the bud length exceeds the seed length 1/2 or the germ length ≥ 2 mm, and the germination is stopped on the 10th day and the seed germination rate is calculated.

2.3 Test measurement index

Germination rate = 10 d number of germinated seeds / number of tested seeds × 100%;

Germination potential = 3 d number of germinated seeds/number of tested seeds × 100%;

Relative germination rate: salt treatment germination rate/ control treatment germination rate × 100%;

Relative germination potential: salt treatment germination potential / control treatment germination potential × 100%;

Salt tolerant semi-lethal concentration: salt concentration when the relative germination rate reaches 50%;

Salt-tolerant limit concentration: the salt concentration at a relative germination rate of 10%.

2.4 Data processing

The test data was collated and plotted using Excel, and statistical analysis was performed in SPSS 18.0 software. One-way ANOVA was used to analyze the relationship between seed germination rate, germination potential, relative germination rate and relative germination potential and NaCl concentration by linear regression analysis.

3. Results and analysis

3.1 High concentration NaCl solution inhibited the germination rate of Zhongmu No.3 seed

The cumulative germination rate of alfalfa seeds treated with different concentrations of NaCl is shown in Figure 1. The cumulative germination rate curves of seeds under different treatments are quite different. CK, T1 and T2 were observed in 1 d, T3 and T4 in 2 d, and 6 d in T6. At 2 d after germination, the cumulative germination rates of T1, T2 and T3 decreased by 9.3%, 51.5% and 80.9%, respectively, compared with CK. From the appearance of germination to the stable germination rate, the NaCl concentration was more than 200mmol/L, and the period was sharply shortened (0-1 d). The rest of the treatment period was 4-6 days, and the difference was small. When the NaCl concentration is ≤150 mmol/L, the number of days that reach the cumulative germination rate increases with the increase of NaCl concentration (5-8 d), and the hysteresis effect (4-6 d) occurs after more than 150 mmol/L; The salt stress shortens the number of days to reach the maximum germination rate. With the increase of germination time, the difference between the cumulative germination rate and CK of each treatment gradually decreased, and remained unchanged after the maximum germination rate.

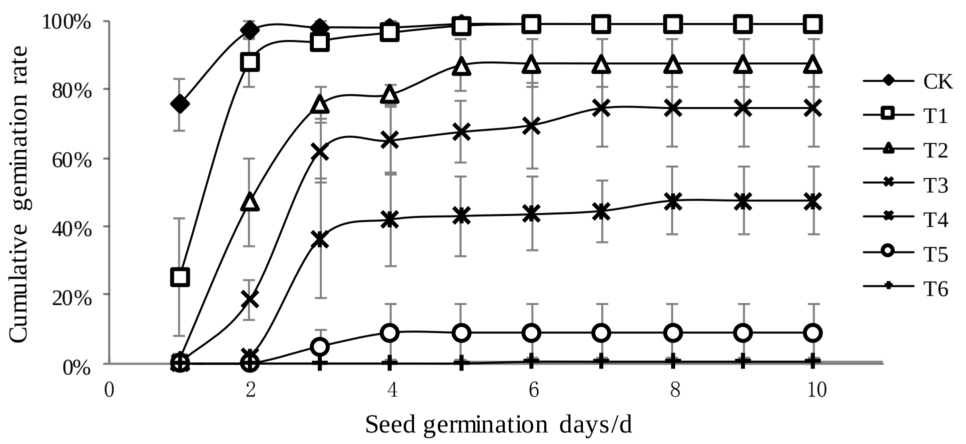

Fig.1 Effect of concentration of NaCl on seed daily germination rate in Medicago sativa

Table 1 shows the variance test table for the germination rate of Zhongmu No. 3 under different concentrations of NaCl. The variance of the data between the treatments does not satisfy the homogeneity hypothesis (P=0.002<0.05), and the variance analysis can be performed by Tamhane's T2 test. The germination rate and one-way analysis of variance of each treatment seed were as shown in Fig. 2. The germination rate of CK was the highest, which was 99%. The germination rate of the seed treated with T6 was the lowest, 0.5%. The germination rate of T1 treated alfalfa seeds was the same as that of CK, while the germination rate of other treated alfalfa seeds decreased significantly with the increase of salt content, and the decline trend after more than 100 mmol/L (T3) was more obvious. The variation of germination rate with NaCl concentration indicates that low concentration (0-50 mmol/L) NaCl treatment has little or no stimulating effect on the germination rate of alfalfa seeds (the germination rate is about 10%), and the high concentration (100- 150 mmol/L NaCl treatment significantly inhibited seed germination. The higher the concentration, the more obvious the inhibition. The excessively high concentration (200-250 mmol/L) NaCl-treated seeds did not germinate.

Tab.1 Homogeneity test of variance of germination rate in Medicago sativa

LeveneStatistics	df1	df2	Significant
5.197	6	21	0.002

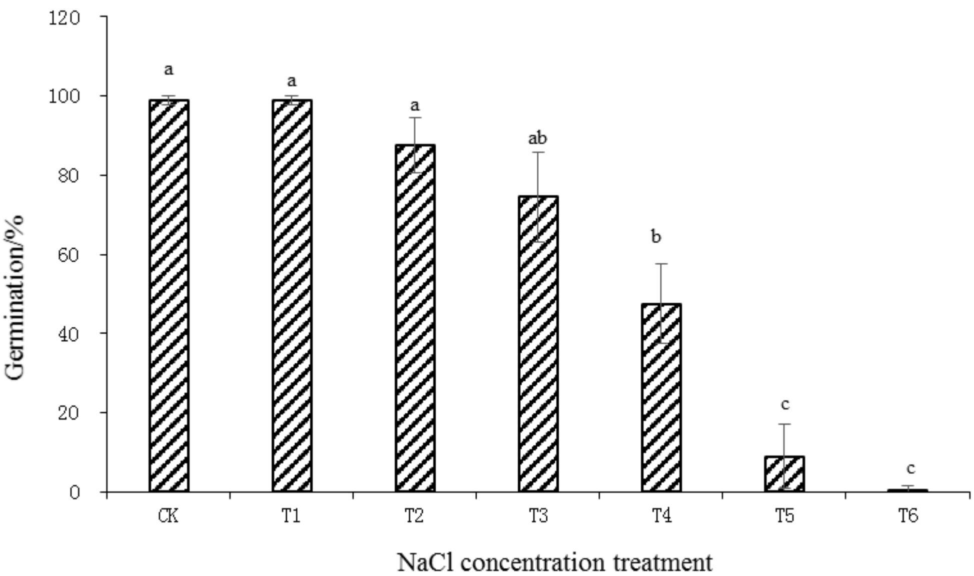

Fig.2 Effect of concentration of NaCl on seed germination rate in Medicago sativa

Note: Different letters within the picture mean significant difference between the treatments at the 5% level. The same as below.

Since the germination rate is unchanged when the NaCl concentration is ≤25mmol/L, the piecewise linear function equation is used to fit the relationship between NaCl concentration and germination rate, so that the germination rate of 0-25 mmol/L is equal to the measured value, as shown in Fig. 3. Shown. The coefficient of determination for the linear fitting equation was 0.97, and the significance test level was P<0.01. The germination rate of NaCl concentration over 25 mmol/L was significantly and linearly negatively correlated with the salt content of the culture solution, and the germination rate decreased by 4.5% for every 10 mmol/L of NaCl concentration until it reached zero at 245 mmol/L. Germination rate.

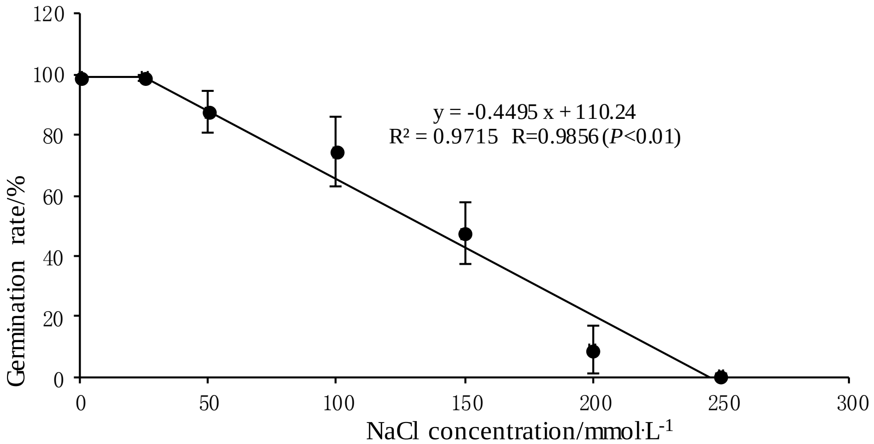

Fig.3 Straight line fitting of NaCl concentration and germination rate

It can be seen from Fig. 4 that when the NaCl concentration of the culture solution is greater than 25 mmol/L, the relative germination rate of Zhongmu No. 3 seed decreases significantly with the increase of NaCl concentration. When the concentration reached 50 mmol/L to 100 mmol/L, the germination rate of Zhongmu No.3showed a steady downward trend, while the relative germination rate decreased significantly when it reached the concentration of 150 mmol/L, so 50 mmol/L (equivalent to There is a limit between the salinity of 2.9 g/L brackish water and 100 mmol/L (equivalent to a salinity of 5.9 g/L salt water). When brackish water is used for irrigation or when the soil salinity is equivalent to less than 5 g/L, the salt concentration has little effect on the germination and germination process of the alfalfa. When the concentration is higher than the limit, the salt ion has a serious effect on it. Suitable for normal germination of Zhongmu No.3. It is indicated that the brackish water (mineralization degree 3-5 g/L) can't cause the germination rate to drop seriously, while the salt content is too high, it is not suitable for the irrigation water source of germination of alfalfa seeds. The piecewise function was used to fit the relationship between relative germination rate and NaCl concentration. According to the fitted regression equation (relative germination rate = -0.454 × NaCl concentration + 111.35), the salt-tolerant LC50 concentration of Zhongmu No.3 was 135 mmol. /L, the salt-tolerant concentration was 223 mmol/L.

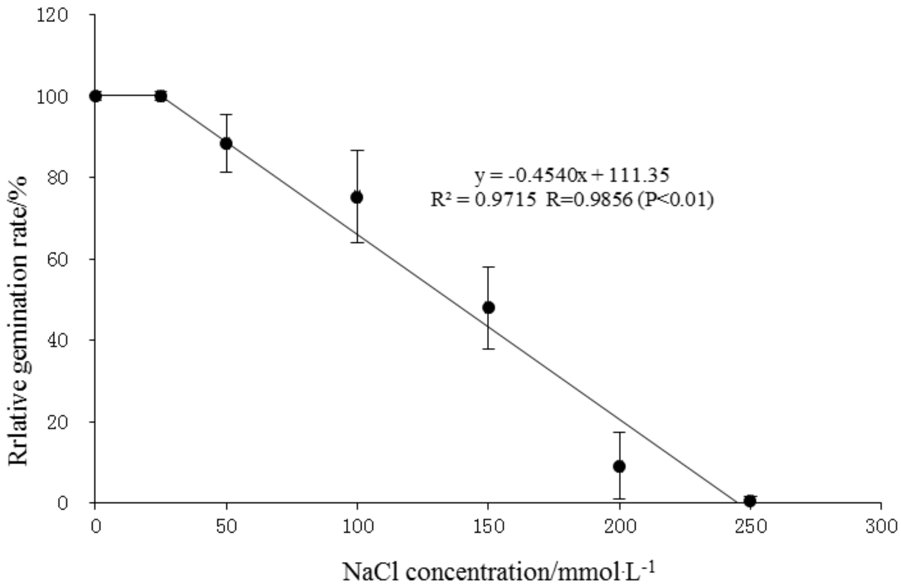

Fig.4 Effect of concentration of NaCl on seed relative germination rate in Medicago sativa

3.2 Effects of Different Concentrations of NaCl on the Germination Potential of Zhongjing No.3 Seeds

Table 2 shows the variance test table for the germination potential of Zhongmu No. 3 under different concentrations of NaCl. The variance of the data between the treatments does not satisfy the homogeneity hypothesis (P=0.002<0.05), and the variance analysis can be performed by Tamhane's T2 test. The germination potential and one-way analysis of variance of each treatment showed that the germination potential of CK was 98%. The germination potential of T6 was 0. Compared with CK, the germination potential of alfalfa seeds decreased by 4.08% compared with CK,94%; under T2 treatment, the germination potential decreased by 22.96%, which was 75.5; the germination potential decreased by 36.73% under the treatment of T3, which was 62%; this indicates that the degree of germination decreased with the increase of NaCl concentration; Low concentration (25 mmol/L) NaCl solution had little effect on the germination potential of alfalfa. At this time, the vigor of the alfalfa seeds was strong, the germination was uniform, and the yield increased. The activity of alfalfa seeds under T2 and T3 treatment was reduced and neat. Sexual decline, but there is still a certain potential for increased production; T5, T6 treated seed loss is lost, no longer have the ability to increase production

Tab.2 Homogeneity test of variance of germination potential in Medicago sativa

LeveneStatistics	df1	df2	Significant
5.197	6	21	0.002

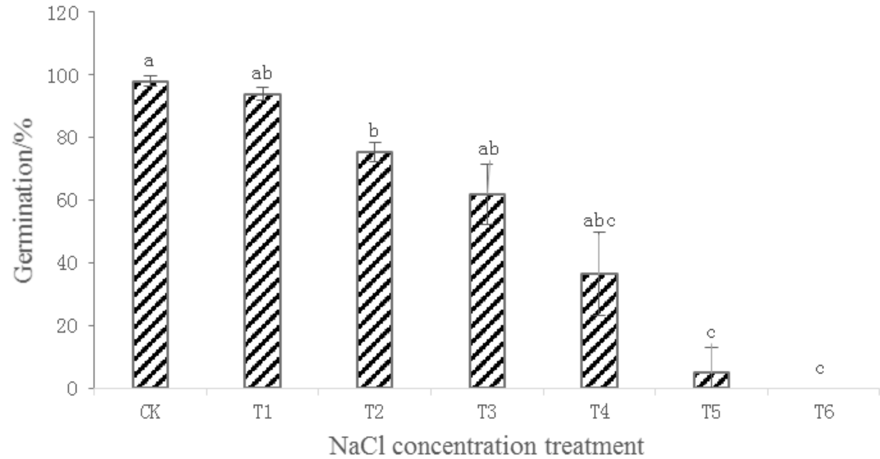

Fig. 5 Effect of different concentrations of NaCl on the germination potential of alfalfa seeds

The relationship between NaCl concentration and germination potential was fitted using a linear function equation, as shown in Figure 6. The coefficient of determination of the linear fitting equation is 0.98, and the significance test level (P <0.01) indicates that the regression equation of this alfalfa variety has a good fit, and the germination potential has a very significant linear negative correlation with the salt content of the culture solution, and the NaCl concentration. For every 10 mmol/L increase, the germination potential decreased by 4.3%.

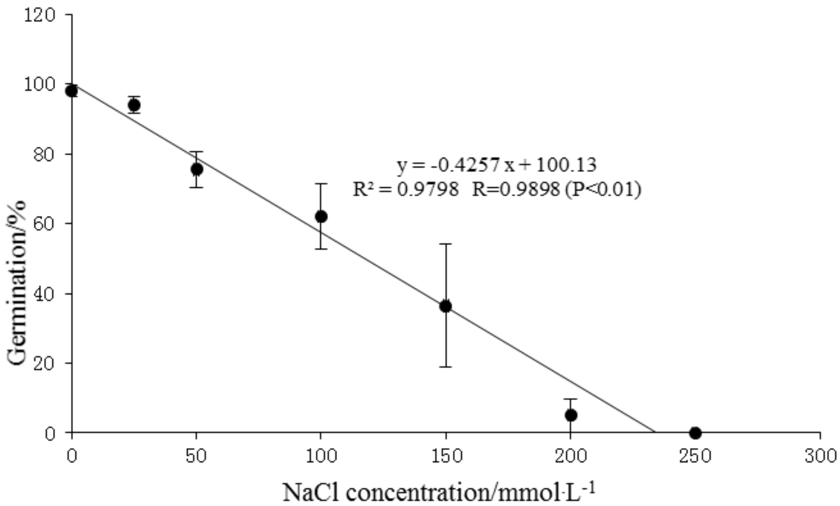

Fig.6 Straight line fitting of NaCl concentration and germination potential

Relative germination potential can indicate the vitality of cockroaches in the early stage of germination. The relative germination potential of the tested ruthenium material decreased with the increase of salt concentration. Under the treatment of 25 mmol/L salt concentration (T1), the average relative germination potential was 95.95%, indicating that a certain concentration of salt stress can stimulate the germination of plant seeds, because the seed germination process also requires a certain ion concentration. Infiltration of ions can activate certain enzymes in the metabolic process, making the seeds necessary for seed germination more complete, thus making germination more rapid; while under salt stress of 200-250 mmol/L, the relative germination potential of the test materials is almost 0, indicating that high concentration of salt stress has an inhibitory effect on germination.

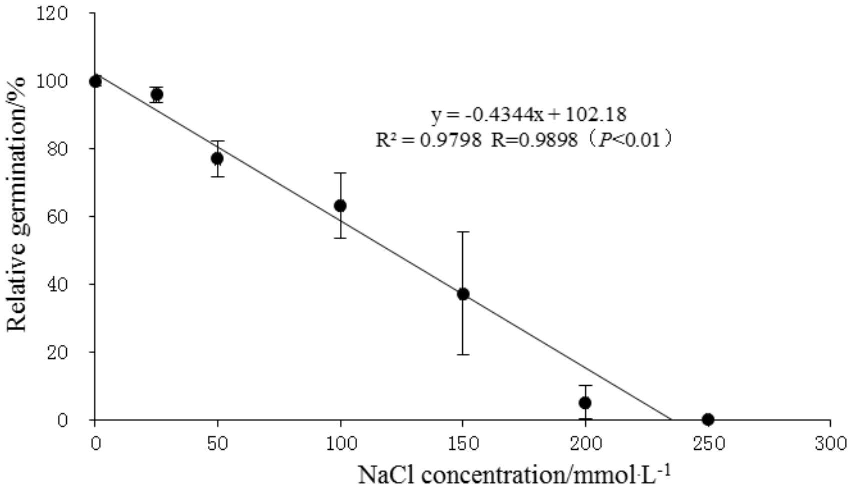

Fig.7 Effect of different concentrations of NaCl on the relative germination potential of alfalfa seeds

4. Discussion and conclusion

The seed germination period is the most sensitive period for plant stress[9]. Whether a plant can survive in a saline-alkali environment depends first on whether it can germinate, and the germination rate and germination potential. The germination rate and germination potential are often used as indicators for evaluating seed germination, which can reflect the germination speed of seeds, and the difference in seed germination is very large. Even if the same variety is derived from one region, the salt tolerance is quite different [10]. The degree of inhibition of seed germination is related to the type and concentration of salt and the salt tolerance of plants. The damage of salt to seed germination has two main aspects: one is the osmotic effect, and the other is the ion effect. The osmotic effect means that when the salt content in the soil increases, the osmotic pressure increases, and the water potential decreases correspondingly, making the seed difficult to absorb water, thereby affecting the germination of the seed; the ion effect means that the ion concentration is increased to poison the seed[11], under the stress of ions, causing intracellular ion imbalance, thereby destroying the normal physiological functions of the cells.

Plant seeds are affected to varying degrees under different salt concentration stresses. The germination rate of seeds of each cultivar was not significantly inhibited under the stress of low concentration of salt solution[12]. Salt tolerance identification is the basis for breeding salt-tolerant varieties [13]. Salt tolerance is a complex physiological process and a comprehensive manifestation of multiple metabolisms. Therefore, the selection of reasonable salt tolerance indicators for salt tolerance identification Especially key[14]. The salt tolerance identification indicators used in most of the researches mainly focus on germination potential, germination rate and other related indicators, and a small number of studies have adopted seedling length and root length [15].

The results of this experiment showed that compared with CK (control), low concentration (25 mmol/L) NaCl salt stress had a stimulating effect on the germination and seedling growth of Zhongmu No.3 seed, and high concentration of salt stress would poison the seed. And inhibit its germination.The high salt solution will cause ion toxicity to the seed and inhibit seed germination. With the increase of salt stress concentration, the germination rate and germination potential of Zhongmu No.3 seed decreased($P<0.05$), and the germination rate and germination potential were significantly negatively correlated with NaCl concentration ($P<0.01$), this test has a salt-lethal lethal concentration of 135 mmol/L, salt-tolerant concentration of 223 mmol / L. At the same time, it was found that the low concentration of salt stress had little effect on the germination potential of alfalfa germination rate. In some cases, it even promoted. With the increase ofNaClconcentration, the inhibitionofalfalfa seed germination increased significantly. Germination becomes sluggish and seed necrosis is achieved at high salt concentrations. To adapt to the environment, seeds should not only be able to germinate, but also depend on whether the seedlings can grow normally. This is for further study in the future.

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