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Effects of Saline-alkali Stress on the Growth of Transgenic Sorghum Overexpressed *SbFLA*15 in Bud Stage

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Abstract | **Original Research Article**

In this study, wild-type sorghum *P*898012 (WT) was used as a control to investigate the effect of salinity stress on the growth of the overexpressed *SbFLA*15 sorghum lines (OE) in bud stage. The results of the concentration screening of saline-alkali stress solution showed that 75 mM ($NaHCO_3$: Na_2CO_3 is 5:1, pH 9.63) was more suitable to simulate stress. Under saline-alkali stress, the germination and growth of different transgenic strains showed different resistance, with OE 1 being the strongest and OE 7 the worst. OE 1 was selected as the research subject to further investigate the impact of saline-alkali stress on the growth of overexpressed *SbFLA*15 sorghum lines during the bud stage, and the results showed that the relative germination potential, bud length, germination index, fresh weight and dry weight of OE 1 were better than those of WT, showing stronger ability to resist saline-alkali stress.

Keywords: Sorghum, *SbFLA*15, Saline-alkali stress, Bud stage, Growth index.

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.)) is a highly adaptable cereal crop with multiple resistance [1] such as salinity and drought resistance, and is able to grow in paddy fields and saline-alkali fields, and even survive in arid and semi-arid areas. It is the fifth largest grain crop [2] in the world. The growth cycle of sorghum typically ranges from 3 to 4 months, and it may vary based on the specific variety and climatic conditions. Sorghum is rich in a variety of nutrients, such as cellulose, carbohydrates, trace tannin, minerals and others [3], with grain, feeding, brewing and other uses. Sorghum has a sweet and mild taste and is slightly cold. It has the effect of cooling blood and detoxifying and has been used medicinally to prevent and treat conditions such as diabetes [4] and ovarian cancer [5].

Soil salinization alters both the physical and chemical properties of the soil, leading to decreased ventilation and water permeability, lowered soil water potential, and restricted water absorption capacity of plants [6]. In the high saline-alkali environment, plants are prone to "burning seedlings", which makes it difficult for plants to absorb water, resulting in physiological drought, and even causing water backflow in root cells, while affecting the growth and development and normal physiological processes such as photosynthesis [7]. Saline-alkali stress will interrupt seed germination through osmotic damage and affect endogenous hormones. Most plants can not maintain the homeostasis of ROS and ions in germinating seeds under saline-alkali stress, thus delaying seed germination due to oxidative and osmotic damage [8].

Arabinogalactan-proteins (AGPs) are a class of hydroxyproline-rich cell wall glycoprotein [9], which are found in cell membranes, cell walls, and extracellular secretions [10], and affect a variety of biological processes such as plant growth, development, reproduction, and abiotic stresses. FLA proteins are one of the subclasses, containing 1-2 fasciclin-like domains (FAS) that are not found in other AGP subfamilies [11]. FLA genes are related to the cell wall [12], which is an important line of defense for plants in contact with the external environment, and plays the roles of structural support [13], material transport and defense against adversity in plant growth and development [14]. Upon stress, plants can respond to stress by triggering organ-specific cell wall remodeling [15] and modifying signals related to cell wall structure and composition [16].

In this study, by analyzing the effects of saline stress on the germination and growth of overexpressed

*SbFLA*15 transgenic sorghum, and preliminarily explored the role of *SbFLA*15 gene in enhancing the saline tolerance of sorghum, so as to provide new candidate genes for the molecular breeding of sorghum to tolerate saline and alkali stresses.

2. THE MATERIALS AND METHODS

WT (wild-type *P*898012) sorghum seeds and OE (overexpressed *SbFLA*15 gene) transgenic sorghum seeds used in this study were provided by the Plant Gene Function Laboratory of Heilongjiang Bayi Agricultural University.

2.1 Seed germination

A number of sorghum seeds with full grains, uniform size and same number of seeds were selected, the soil on the surface of the seeds was washed off with distilled water, placed in 75% alcohol for 2-3 min, then washed 3-5 times with distilled water until there was no smell of alcohol, and then soaked in 3% sodium hypochlorite for 3 min, and washed 5 times with distilled water. The sorghum seeds of each strain were placed into petri dishes separately, 10 mL of distilled water was added, and the seeds were germinated in the dark for 24 h. The seeds were then washed with 3% sodium hypochlorite for 3 min and distilled water for 5 times.

After the seeds germinated for 24 h, the seeds were transferred to a Petri dish covered with double-layer filter paper, with 50 seeds in each dish and repeated 3 times in each group. WT and OE seeds were treated with distilled water (providing environmental conditions for sorghum seed germination) or Hoagland nutrient solution (providing nutritional conditions for sorghum seed growth) as control group (control), and saline-alkali stress solutions (50 mM, 75 mM, 100 mM and 150 mM) with different concentrations were prepared with distilled water or Hoagland nutrient solution for stress treatment as experimental group (treated), and the solution was changed every 1 d. The optimum concentration of saline and alkaline stress for the growth of sorghum was screened, and the growth morphology and growth indexes were recorded.

2.2 Determination of sorghum growth indicators

A seed whose roots and buds are longer than 1/2 of the seed is recorded as germination. The number of germinated seeds on the first, third and fifth day of germination was recorded, and the corresponding germination index was calculated and photographed. Germination rate (GR) = $G_5/N\times100\%$(G_5 is the number of seeds germinated on the fifth day, and N is the number of seeds tested); Germination potential (GP) = $G_3/N\times100\%$(G_3 is the number of seeds germinated on the third day); Relative germination potential (%) = germination potential of stress group/germination potential of control group×100%; Germination index (GI) =$\Sigma G_t/D_t$ (D_t indicates the corresponding germination days, and G_t indicates the number of germinated seeds per day corresponding to D_t); Relative germination index (%) = germination index of stress group/germination index of control group× 100%.

Bud length was measured and the data recorded. The fresh weight of the buds was determined using a balance, after which they were dried in an oven at 65°C to a constant weight, and their dry weight was determined and the data recorded.

2.3 Data Processing

Statistical analysis of the data was performed using IBM SPSS & Statistics 20.0 software. Significance analysis was performed using independent sample t-tests to compare the effects of WT and OE parameters, and $P < 0.05$ indicates a statistical difference, and the results were graphed using Prism 8 software.

3. RESULT ANALYSIS

3.1 Screening of saline-alkali stress solution concentration

In order to explore the concentration of saline-alkali stress solution used in the experiment, WT, OE 1, OE 2, OE 3 sorghum seeds were selected, the control group was treated with distilled water, and the experimental group was treated with 50 mM, 75 mM, 100 mM, 150 mM saline-alkali stress solution. The growth after 3 days of culture was shown in Figure 1.

When the concentration of saline-alkali stress solution was 50 mM, there was no significant difference in the growth morphology of the four strains WT, OE 1, OE 2, and OE 3; When it was 75 mM, the growth morphology of the four strains was obviously different; At other concentrations, the growth morphology of the four strains appeared to be different, but the seed germination rate was too low and the bud length was significantly shorter. It was difficult to sample and measure growth indicators for subsequent experiments. After comprehensive consideration, 75 mM stress solution was chosen to simulate a saline-alkali stress environment in subsequent experiments.

3.2 Screening of saline-alkali resistant *SbFLA*15 sorghum strains

In order to screen the sorghum strains with the strongest resistance to saline-alkali stress, WT, OE 1, OE 2, OE 3, OE 4, OE 5, OE 6, OE 7, and OE 8 sorghum seeds were selected for control and salt-alkali stress treatment and growth after 6 days of culture was as shown in Figure 2. Among the eight OE lines, OE 1 showed the most significant difference in growth form under saline stress compared with WT, and the OE 1 line was selected for subsequent experiments.

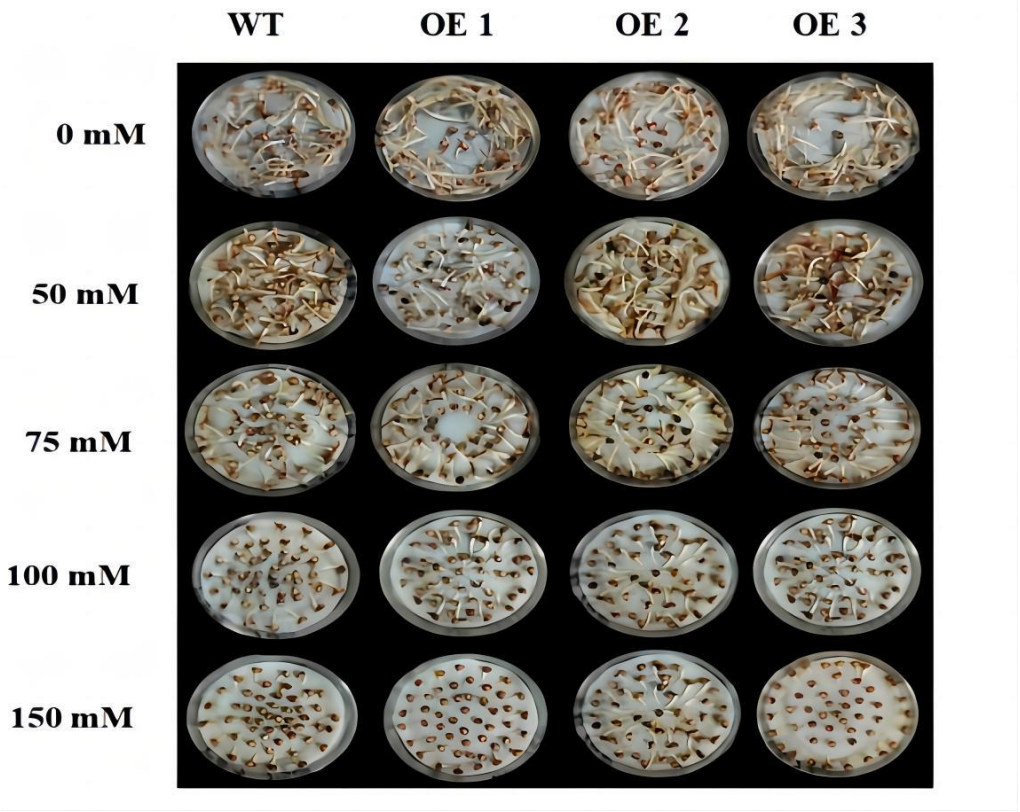

Figure 1: Growth status of WT, OE 1, OE 2, and OE 3 on 3 d (under different concentrations of saline-alkali stress)

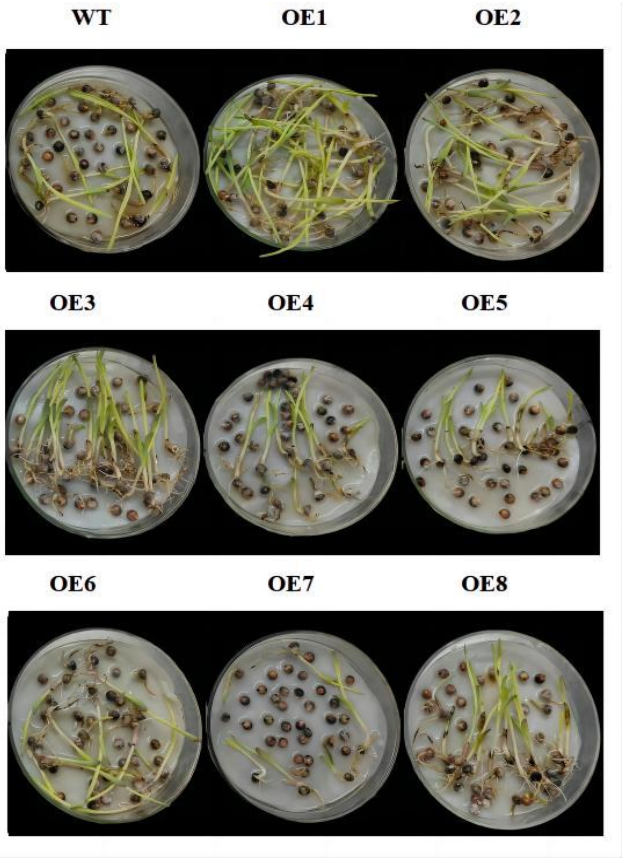

Figure 2: Growth situation of WT, and overexpressed sorghum lines on 6 d

3.3 Effects of saline-alkalistress on germination indicators in *SbFLA*15 transgenic shoots

By the fifth day, the germination index of WT and OE 1 were shown in Figure 3. Saline-alkali stress inhibited sorghum germination rate, with differing degrees of inhibition observed between WT and OE 1. The relative germination potential of OE 1 was under salinity stress significantly higher than that of WT, indicating that the degree of damage to OE 1 under saline-alkali stress was less than that of WT.

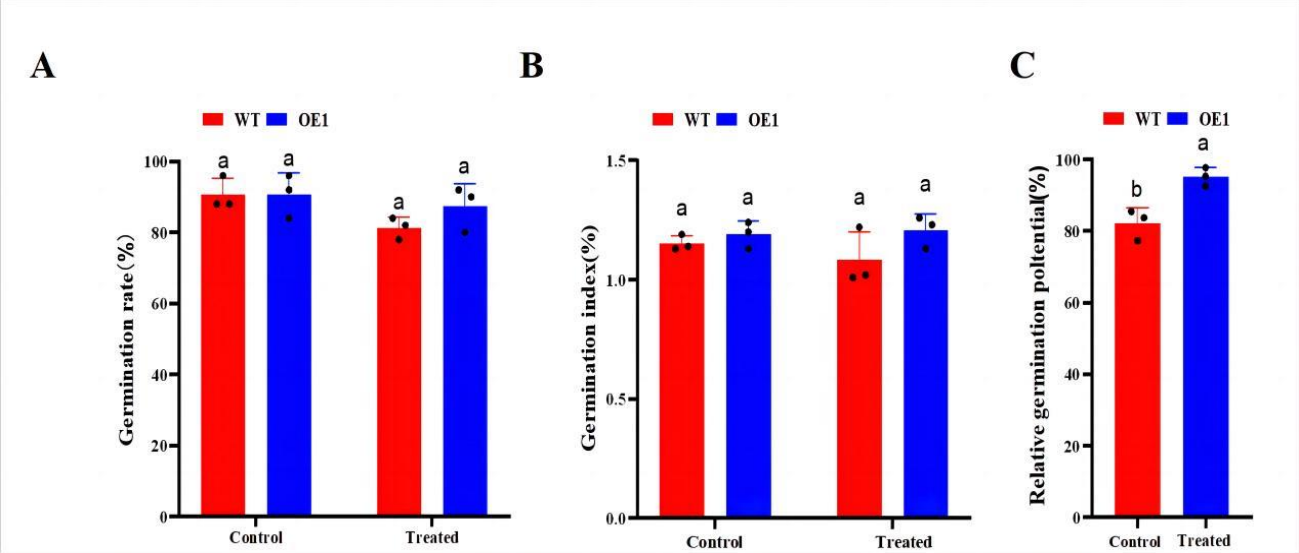

Figure 3: The effects of saline-alkali stress on germination indicators of WT and OE 1 (Figure A shows the effect on germination rate, Figure B shows the effect on germination index, Figure C shows the effect on relative germination potential, $P < 0.05$)

3.4 Effects of saline-alkali stress on germination of *SbFLA*15 transgenic sorghum seeds

After cleaning and disinfection, several WT and OE 1 seeds were selected, and the control group germinated in distilled water, while the experimental group germinated in 75 mM saline-alkali stress solution prepared by distilled water. After 24 hours of germination, the control group was cultured with nutrient solution and the experimental group was treated with 75 mM saline-alkali stress solution prepared with nutrient solution. On the fifth day of germination, the germination of WT and OE1 was as shown in Figure 4. Under nutrient solution treatment, the growth state of OE 1 is better than that of WT; under the treatment of 75 mM saline-alkali stress solution, the germination and growth state of OE 1 were better than that of WT. As can be seen from Figure 4, compared with the control group, the germination number of sorghum under saline-alkali stress decreased sharply, the germination rate decreased, and the growth status of leaves became obviously worse, while the germination number of OE 1 under saline-alkali stress was significantly higher than that of WT.

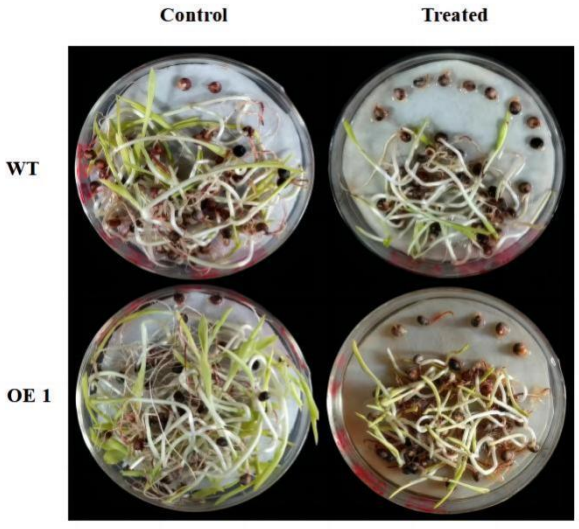

Figure 4: Germination status of WT and OE1 on 5 d (germinated seeds in each plate are placed at the bottom, and germinated seeds are placed at the top)

3.5 Effects of saline-alkali stress on growth of *SbFLA*15 transgenic sorghum at bud stage

3.5.1 Effects on growth morphology of *SbFLA*15 transgenic sorghum at bud stage

On the fifth day of germination, the growth morphology of WT and OE 1 was shown in Figure 5. Compared with the control group, the sorghum seedlings after saline-alkali stress became significantly shorter, the young leaves gradually became wilted, curled, or even yellow, and the roots were reduced and shortened. The seedling length, leaf color, root and other growth conditions of OE 1 under saline-alkali stress was significantly better than those of WT, indicating that the impact of saline-alkali stress on OE 1 is less than that of WT, and the growth morphology of the OE 1 experimental group is better than that of WT.

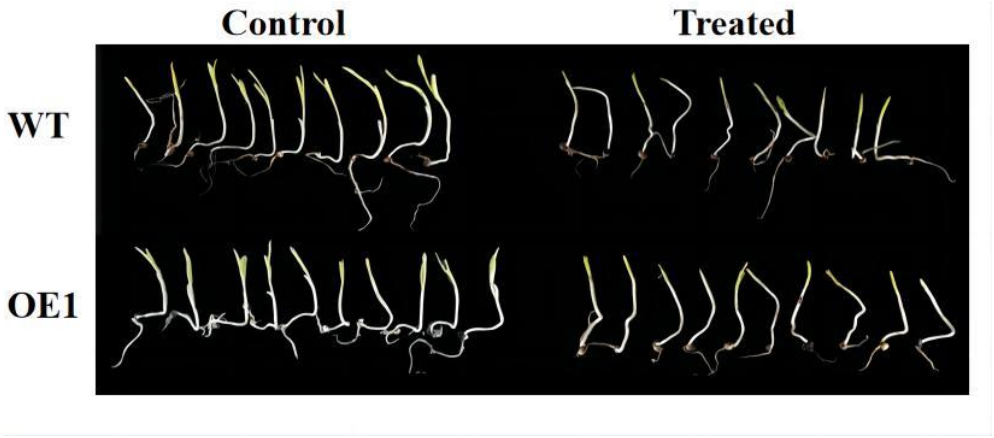

Figure 5: Growth morphology of WT and OE 1 on 5 d (the left column is the nutrient solution treatment, the right column is the 75 mM saline-alkali stress solution treatment, the first row is WT, the second row is OE 1)

3.5.2 Effects on *SbFLA*15 sorghum bud length

On the fifth day of germination, the bud length of WT and OE 1 was shown in Figure 6. The bud length range of the control group of WT was 5-10 cm, the bud length range of the experimental group was 2-5 cm, the bud length range of the OE 1 control group was 5-9 cm, the bud length range of the experimental group was 4-6 cm, saline-alkali Under stress, the overall shoot length of OE 1 was longer than that of WT. Compared with the control group, the sorghum bud length after saline-alkali stress equail decreased, the seed shoots decreased overall, and WT shoots decreased significantly, which was significantly lower than OE 1, indicating that OE 1 was more salinity-tolerant than WT.

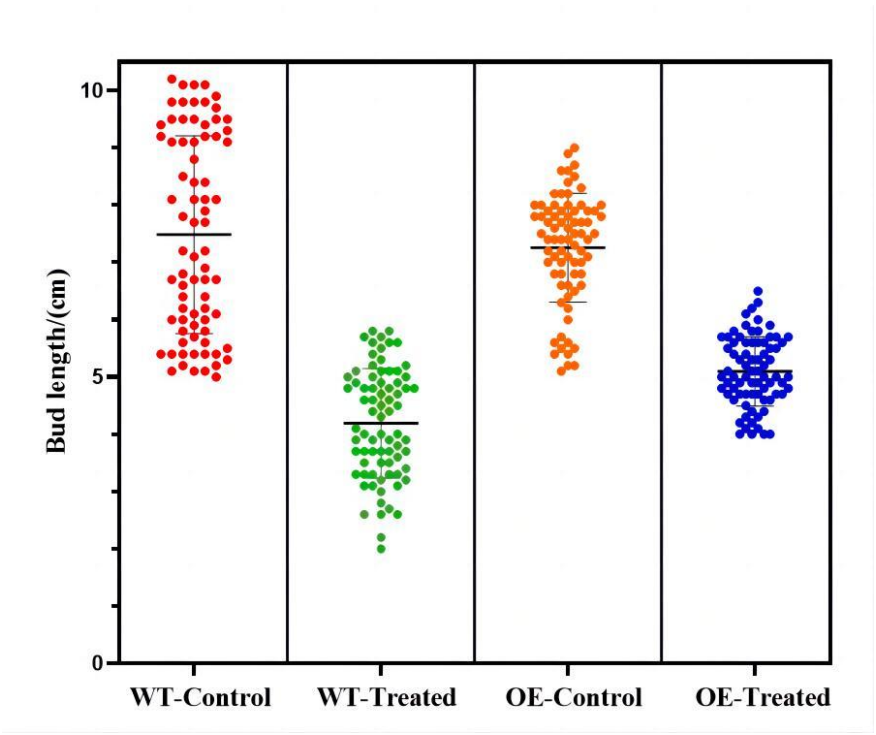

Figure 6: Effects of saline-alkali stress on bud length of WT and OE 1

3.5.3 Effects on *SbFLA*15 sorghum bud fresh weight and dry bud weight.

On the fifth day of germination, the fresh weight and dry weight of WT and OE 1 buds were shown in Figure 7. Under saline-alkali stress, the bud fresh weight and dry bud weight of WT and OE 1 decreased. The fresh weight of WT and OE 1 buds decreased significantly under saline-alkali stress, the dry weight of WT buds decreased significantly under saline-alkali stress, and the dry weight of OE 1 buds did not decrease significantly under saline-alkali stress, indicating that OE 1 was more tolerant to saline-alkali than WT.

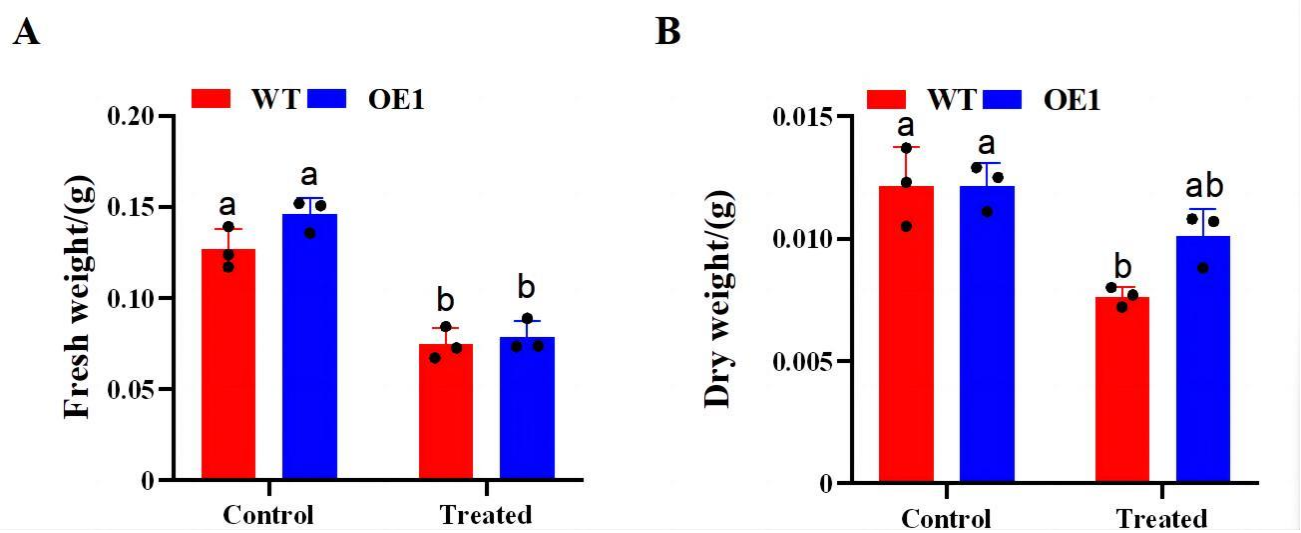

Figure 7: Effects of saline-alkali stress on the fresh weight and dry weight of buds in WT and OE 1 (Figure A shows the effect on the fresh weight of buds, Figure B shows the effect on dry weight of buds, $P < 0.05$)

4. DISCUSSION

Seed germination, as the initial stage of plant growth and development, is the period during which plants are most vulnerable to various adversity stresses [17], which determines the number of basic seedlings in the whole field by affecting seedling emergence and seedling success, and ultimately affects yield composition [18]. Germination rate, germination potential, germination index and other indicators reflect the seed germination rate, germination neatness and seedling robustness potential, which are commonly measured indicators for detecting seed quality [19-21]. In addition, some scholars have also identified the salinity tolerance of sorghum seedlings, measuring the seedling height, maximum leaf area of young leaves, root length, chlorophyll content and other indicators [22]. Taken together, the germination rate, germination index, relative germination potential, shoot length, fresh weight, and dry weight at germination stage were selected as the identification indexes of salinity tolerance of sorghum in this study.

Appropriate screening concentration can maximize the coefficient of variation and the range of variation of each index among materials. In previous studies, the concentration of saline-alkali stress treatment was mainly 100 mM, 150 mM, 200 mM and 300 mM, and the solutes were NaCl, Na_2SO_4 and $NaHCO_3$ [22-23]. In this experiment, a finer concentration gradient was set between 50-150 mM, and the growth index of the experimental materials was measured in 75 mM saline-alkali stress solution, and the soda saline-alkali stress environment was simulated with $NaHCO_3$ and Na_2CO_3.

The FLA gene family has been studied and proven to play important roles in a variety of plants, *GoFLA*19*s* are associated with male sterility in cotton [24], *FLA*16 regulates *Arabidopsis thaliana* stem development [25], and in tomato, *SbFLAs* show a positive response to salt and cold stresses [26]. The FLA gene family has seldom been reported in sorghum, and in the present study, overexpression of *SbFLA*15 the relative germination potential, shoot length, and shoot dry weight of sorghum under saline and alkaline stress at the shoot stage were better than those of wild type, which provided data support for further research on the mechanism of *SbFLA*15 in regulating the tolerance of sorghum to saline and alkaline stress, as well as new genes for the molecular breeding of sorghum to tolerate saline and alkaline stress, and may be used for breeding new varieties to improve the yield of sorghum under unfavourable environmental conditions. This study was limited to the effect of saline stress on sorghum at shoot stage, and there is no relevant study on the effect at seedling stage. The next step is to plan to study the effect of saline stress on sorghum at seedling stage.

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