

# Evaluation of saline tolerance at the seedling stage of various maize cross combinations

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## **Abstract:**

This study was conducted at the Maize Research Institute of Vietnam using 24 maize hybrids, identified as STM1 to STM24, at five different salt concentrations, which were 0, 50, 100, 150, and 200 mM NaCl. After seven days of germination at room temperature, seedlings were transplanted to nutrient-rich solutions salinized with salt. 17 day-old seedlings were then harvested to calculate their growth parameters of the day to seedlings fully wilt and degradation, root and shoot length, seedling dry matter production, and content. As the study results show, salt stress caused the reduction of plant growth in all hybrids, however, the STM10 and STM21 varieties showed the best salt tolerance, while STM17 and STM18 presented most salt-sensitive. The analyzed results for Na<sup>+</sup> and K<sup>+</sup> showed that among the hybrids, STM21 had lower Na<sup>+</sup> and higher K<sup>+</sup> contents in both roots and shoots of the seedlings than others.

**Keywords:** maize hybrid, salt tolerance, seedling.

**Classification number:** 3.1

## **Introduction**

Salinization is the accumulation of salt in the soil, which strongly influences the agricultural production, the health of the environment, and the economy of the country [1]. According to Wild (2003), about 15% of the land in the world has been eroded, and the physical and chemical characteristics of the Earth's soil have been modified by various factors, including soil salinization [2].

In Vietnam, saline soils are formed primarily from salt or salt-water moving in from the sea or from underground movements of salt sources up to the soil surface. Another reason for soil salinity is saline water used to water fields because of a lack of fresh water. Evaporation during crop farming also causes soil surface salinization. There are about three million hectares of land with high salinization and acidification distributed mainly

across the provinces of the Mekong River Delta, including in the areas of: Bac Lieu, Ca Mau, Kien Giang, Tra Vinh, and Ben Tre; and the provinces of the Red River Delta, including: Quang Ninh, Hai Phong, Thai Binh, Nam Dinh, Ninh Binh, and Thanh Hoa [3].

Most crops tolerate salinity to a threshold level, above which salt levels will begin to decrease crop productivity [4]. Maize is a pollinated crop and is able to tolerate salinity [5]. Research into maize genotypes is needed to determine materials for maize breeding programs to produce high yield varieties with salt tolerance. Studies into the salt tolerances of maize have been published by scientists [6-10], and have given a scientific basis for salinity tolerance in maize. In Vietnam, salt tolerance in maize has not been studied yet. Facing the evolution of adverse climate change and the increase of the salinization of agricultural land, research of breeding salt-tolerant crops in general, and maize in particular, is an urgent matter to aid in the development of agriculture. Studies of the saline tolerance of maize in fields are very difficult because of the heterogeneity of soil's physical and chemical properties, and fluctuations in seasonal rainfall. Therefore, we conducted the "Evaluation of salt tolerance ability on maize hybrids at the seedling stage" using an objective assessment of maize hybrids for salt tolerance.

## **Materials and methods**

### *Materials*

The research was conducted at MRI (Dan Phuong, Hanoi) using materials from 24 maize crosses: STM1, STM2, STM3... STM24.

### *Methods*

The maize seeds were soaked in distilled water and germinated in sand medium in individual lines for five to seven days at room temperature, and then transplanted to a Yoshida nutritional solution, which was salinized with salt (NaCl) at four concentrations: 50, 100, 150, and 200 mM, including a control of 0 mM. Additional salt was applied in the small amount at 50 mM every five days after transplanting, and the remaining salt was provided after seven days. The nutritional solution was changed twice per week. The experiment was arranged in a randomized complete block design (RCBD) with three replications. After 17 days, the plants were harvested separately in line and treated. Harvested plants were washed in tap water and two times with distilled water, and the following observation parameters were recorded:

*Survival date and salt tolerance ability:* The times from when the plants were transplanted until their deaths in the saline solution.

*Shoot length:* Five plants were taken for plants measurement; the length between the collar and the tip of the longest leaf were measured in cm, and the mean value was reported as the shoot length.

*Root length:* The plants were measured for shoot length and

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were used for root length measurements. The length between the collar and the tip of the longest root was measured in cm, and mean value was reported as the root length.

**Plant dry weight:** Five plants were used for the shoot and root length measurement and were first dried under shaded conditions for some time, and then dried in a hot oven maintained at  $70^{\circ}\text{C} \pm 2$  for 48 hours, and then cooled and weighed. The plant dry weight was expressed in gram/plant.

**Salt Tolerant Index (STI):**

$$\text{STI (\%)} = \frac{\text{Total plant dry weight at treatment } S_x}{\text{Total plant dry weight at treatment } S_0} \times 100$$

$S_x$ : Treatment at salt concentration x;  $S_0$ : Treatment at salt concentration 0.

**Ion  $\text{Na}^+$ ,  $\text{K}^+$  accumulation:** Ion  $\text{Na}^+$ ,  $\text{K}^+$  accumulation was measured using a flame photometer.

**Statistical analysis:** Data showing the variances of factorial design was analyzed using three replications using IRRISTAT statistical software.

## Results and discussions

### Survival date and salt tolerance ability

The observations showed that all crosses reduced growth and wilt after transplanting into the saline solution; this presented very differently between the hybrids. In the S4 treatment, the plants yielded the most degradation without the death plants until 17 days of culture in a nutritional solution including salt. Among them, STM21 showed the highest tolerance in comparison to others (Fig. 1).



**Fig. 1. The effect of salinity on different maize crosses after 17 days of transplanting in 200 mM NaCl (S4).**

### Shoot length

The data in Table 1 shows that the shoot lengths of all crosses reduced when grown in saline solution. In the comparison between S4 (200 mM NaCl) and S0 (0 mM NaCl), STM17 presented the highest reduction of shoot length percentage (45.78%) followed by STM18 (43.98%), whereas the decrease that was the lowest was in STM21 with only 19.72%. The average shoot length of all hybrids decreased by 36.05% when grown in nutritional solution with 200 mM NaCl. It

demonstrated that the salinity of the soil made a great impact on the growth of plants. This was also founded by Carlos, et al. (2007) and André, et al. (2004), when they researched into salt tolerance in maize [6, 11].

**Table 1. The effect of salinity on shoot length of different maize crosses at the seedling stage.**

| No.         | Crosses | Shoot length (cm)               |                    |                     |                     |                     | Mean              | Reduced* (%) |
|-------------|---------|---------------------------------|--------------------|---------------------|---------------------|---------------------|-------------------|--------------|
|             |         | Treatments                      |                    |                     |                     |                     |                   |              |
|             |         | S0<br>(0 mM NaCl)               | S1<br>(50 mM NaCl) | S2<br>(100 mM NaCl) | S3<br>(150 mM NaCl) | S4<br>(200 mM NaCl) |                   |              |
| 1           | STM1    | 31.57                           | 31.03              | 30.03               | 25.73               | 20.13               | 27.70             | 36.22        |
| 2           | STM2    | 35.67                           | 33.73              | 32.18               | 27.48               | 24.47               | 30.71             | 31.40        |
| 3           | STM3    | 33.17                           | 32.87              | 31.87               | 27.57               | 21.63               | 29.42             | 34.77        |
| 4           | STM4    | 34.02                           | 32.62              | 32.72               | 27.30               | 20.88               | 29.51             | 38.61        |
| 5           | STM5    | 31.97                           | 31.67              | 30.67               | 25.97               | 19.37               | 27.93             | 39.42        |
| 6           | STM6    | 30.55                           | 29.47              | 28.47               | 23.77               | 17.17               | 25.88             | 43.81        |
| 7           | STM7    | 32.60                           | 32.30              | 31.30               | 26.70               | 20.10               | 28.60             | 38.34        |
| 8           | STM8    | 33.80                           | 33.50              | 31.33               | 27.97               | 21.37               | 29.59             | 36.78        |
| 9           | STM9    | 33.80                           | 32.67              | 31.33               | 26.87               | 21.70               | 29.27             | 35.80        |
| 10          | STM10   | 33.48                           | 33.18              | 34.37               | 28.47               | 25.10               | 30.92             | 25.04        |
| 11          | STM11   | 34.40                           | 32.53              | 31.53               | 27.67               | 22.80               | 29.79             | 33.72        |
| 12          | STM12   | 32.93                           | 32.63              | 31.63               | 27.33               | 21.33               | 29.17             | 35.22        |
| 13          | STM13   | 33.73                           | 33.43              | 33.53               | 28.00               | 25.02               | 30.74             | 25.84        |
| 14          | STM14   | 29.87                           | 29.57              | 28.57               | 23.87               | 17.27               | 25.83             | 42.19        |
| 15          | STM15   | 31.53                           | 31.23              | 30.23               | 25.53               | 18.93               | 27.49             | 39.96        |
| 16          | STM16   | 34.50                           | 32.67              | 33.20               | 25.93               | 21.90               | 29.64             | 36.52        |
| 17          | STM17   | 33.20                           | 31.40              | 28.90               | 22.33               | 18.00               | 26.77             | 45.78        |
| 18          | STM18   | 31.13                           | 29.90              | 28.90               | 23.00               | 17.60               | 26.11             | 43.98        |
| 19          | STM19   | 32.07                           | 31.77              | 30.77               | 26.07               | 20.63               | 28.26             | 35.66        |
| 20          | STM20   | 34.20                           | 31.23              | 30.23               | 25.40               | 20.93               | 28.40             | 38.79        |
| 21          | STM21   | 33.13                           | 33.90              | 32.90               | 28.80               | 26.60               | 31.07             | 19.72        |
| 22          | STM22   | 32.27                           | 31.97              | 30.97               | 25.13               | 22.30               | 28.53             | 30.89        |
| 23          | STM23   | 32.68                           | 32.38              | 31.38               | 23.63               | 21.80               | 28.38             | 33.30        |
| 24          | STM24   | 27.13                           | 26.83              | 25.83               | 21.13               | 15.20               | 23.23             | 43.47        |
| <b>Mean</b> |         | 32.64                           | 31.85              | 30.95               | 25.90               | 20.93               | 28.46             | 36.05        |
|             |         | <i>LSD<sub>0.05</sub>: 3.49</i> |                    |                     |                     |                     | <i>CV%: 17.6%</i> |              |

\*Reduced shoot length in the comparison between S4 to S0.

### Root length

Root length also was affected by salinity, shown by increasing salt concentrations that reduced root growth (Table 2) and which positively decreased when increasing amounts of salt concentration. However, the response of maize hybrids to salinity was different. Results showed that the average root length of STM18 was the shortest (9.53 cm), while STM10 was at 16.93 cm. However, reductions of root length between the S0 (0 mM NaCl) and S4 (200 mM NaCl) treatments were seen, and the STM21 was reduced the lowest at 18.15%. Studies of maize in salt stress by Khan and Mcneilly (2005) also showed that maize can be grown in saline conditions, but root length decreases rapidly with increasing salt concentration [12].

**Table 2. The effect of salinity on root length of different maize crosses at the seedling stage.**

| No. | Crosses     | Root length (cm)                |                    |                     |                     |                     | Mean  | Reduced* (%) |
|-----|-------------|---------------------------------|--------------------|---------------------|---------------------|---------------------|-------|--------------|
|     |             | Treatments                      |                    |                     |                     |                     |       |              |
|     |             | S0<br>(0 mM NaCl)               | S1<br>(50 mM NaCl) | S2<br>(100 mM NaCl) | S3<br>(150 mM NaCl) | S4<br>(200 mM NaCl) |       |              |
| 1   | STM1        | 17.87                           | 17.67              | 16.67               | 14.87               | 12.87               | 15.99 | 27.99        |
| 2   | STM2        | 16.07                           | 15.87              | 14.87               | 13.07               | 11.07               | 14.19 | 31.12        |
| 3   | STM3        | 16.80                           | 16.60              | 15.60               | 13.80               | 11.73               | 14.91 | 30.16        |
| 4   | STM4        | 17.82                           | 17.62              | 16.62               | 15.15               | 14.22               | 16.28 | 20.21        |
| 5   | STM5        | 19.10                           | 18.90              | 16.83               | 14.63               | 13.90               | 16.67 | 27.23        |
| 6   | STM6        | 16.20                           | 16.00              | 15.00               | 13.20               | 11.00               | 14.28 | 32.10        |
| 7   | STM7        | 18.67                           | 18.47              | 16.23               | 15.93               | 13.73               | 16.61 | 26.43        |
| 8   | STM8        | 15.93                           | 19.60              | 14.73               | 13.47               | 11.33               | 15.01 | 28.87        |
| 9   | STM9        | 18.60                           | 18.40              | 16.80               | 14.60               | 13.67               | 16.41 | 26.52        |
| 10  | STM10       | 19.80                           | 15.73              | 18.33               | 16.27               | 14.53               | 16.93 | 26.60        |
| 11  | STM11       | 16.20                           | 16.00              | 15.00               | 13.40               | 11.53               | 14.43 | 28.81        |
| 12  | STM12       | 17.93                           | 17.73              | 16.73               | 15.07               | 13.07               | 16.11 | 27.14        |
| 13  | STM13       | 18.25                           | 18.05              | 17.05               | 16.00               | 14.38               | 16.75 | 21.19        |
| 14  | STM14       | 17.23                           | 17.03              | 16.03               | 14.23               | 12.03               | 15.31 | 30.17        |
| 15  | STM15       | 17.05                           | 16.85              | 15.85               | 14.05               | 11.85               | 15.13 | 30.50        |
| 16  | STM16       | 16.73                           | 16.53              | 15.53               | 13.73               | 11.53               | 14.81 | 31.08        |
| 17  | STM17       | 16.75                           | 16.55              | 14.82               | 12.62               | 10.48               | 14.24 | 37.41        |
| 18  | STM18       | 17.20                           | 17.00              | 14.47               | 12.20               | 9.53                | 14.08 | 44.57        |
| 19  | STM19       | 16.07                           | 15.87              | 14.87               | 13.07               | 11.80               | 14.33 | 26.56        |
| 20  | STM20       | 18.00                           | 17.80              | 15.87               | 15.20               | 13.33               | 16.04 | 25.93        |
| 21  | STM21       | 18.00                           | 17.80              | 18.20               | 16.33               | 14.73               | 17.01 | 18.15        |
| 22  | STM22       | 17.87                           | 17.67              | 16.33               | 15.27               | 13.87               | 16.20 | 22.39        |
| 23  | STM23       | 16.23                           | 16.03              | 15.03               | 13.23               | 12.07               | 14.52 | 25.67        |
| 24  | STM24       | 16.58                           | 16.38              | 15.38               | 13.58               | 11.38               | 14.66 | 31.36        |
|     | <b>Mean</b> | 17.37                           | 17.17              | 15.95               | 14.29               | 12.49               | 15.45 | 28.26        |
|     |             | <i>LSD<sub>0.05</sub>: 3.42</i> |                    |                     | <i>CV%: 13.80%</i>  |                     |       |              |

\*Reduced root length in the comparison between S4 to S0.

### Plant dry weight

As seen with shoot length and root length, the dry weight of the seedlings decreased with increasing salt concentrations. The average plant dry weight of all crosses at S0 treatment was 0.535 gram/plant, however, it decreased to 0.448 gram/plant at S4 concentration. The dry weight of the seedlings in saline conditions obtained was very different between crosses; STM21 showed the highest dry weight at 0.521 gram/plant, while the lowest observed was in STM17 (0.404 gram/plant). The crosses which presented high plant dry weights in salt concentrations were seen to have high salt tolerant indexes. The results showed that STM21, STM10, and STM13 were

**Table 3. The effect of salinity on plant dry weight of different maize crosses at the seedling stage.**

| No. | Crosses     | Plant dry weight (gram/plant)    |                    |                     |                     |                     | Mean  | Salt tolerant index (%) |
|-----|-------------|----------------------------------|--------------------|---------------------|---------------------|---------------------|-------|-------------------------|
|     |             | Treatments                       |                    |                     |                     |                     |       |                         |
|     |             | S0<br>(0 mM NaCl)                | S1<br>(50 mM NaCl) | S2<br>(100 mM NaCl) | S3<br>(150 mM NaCl) | S4<br>(200 mM NaCl) |       |                         |
| 1   | STM1        | 0.502                            | 0.491              | 0.451               | 0.425               | 0.371               | 0.448 | 73.79                   |
| 2   | STM2        | 0.547                            | 0.508              | 0.469               | 0.441               | 0.387               | 0.470 | 70.81                   |
| 3   | STM3        | 0.515                            | 0.476              | 0.427               | 0.396               | 0.341               | 0.431 | 66.19                   |
| 4   | STM4        | 0.503                            | 0.465              | 0.419               | 0.424               | 0.317               | 0.425 | 63.00                   |
| 5   | STM5        | 0.490                            | 0.499              | 0.440               | 0.392               | 0.362               | 0.437 | 73.93                   |
| 6   | STM6        | 0.569                            | 0.529              | 0.468               | 0.422               | 0.349               | 0.467 | 61.34                   |
| 7   | STM7        | 0.528                            | 0.490              | 0.441               | 0.405               | 0.339               | 0.441 | 64.20                   |
| 8   | STM8        | 0.501                            | 0.460              | 0.414               | 0.380               | 0.312               | 0.413 | 62.28                   |
| 9   | STM9        | 0.487                            | 0.513              | 0.468               | 0.373               | 0.300               | 0.428 | 61.58                   |
| 10  | STM10       | 0.577                            | 0.536              | 0.509               | 0.491               | 0.450               | 0.513 | 77.94                   |
| 11  | STM11       | 0.528                            | 0.490              | 0.439               | 0.405               | 0.344               | 0.441 | 65.15                   |
| 12  | STM12       | 0.548                            | 0.510              | 0.460               | 0.419               | 0.358               | 0.459 | 65.27                   |
| 13  | STM13       | 0.560                            | 0.525              | 0.498               | 0.487               | 0.442               | 0.503 | 78.99                   |
| 14  | STM14       | 0.515                            | 0.469              | 0.408               | 0.362               | 0.317               | 0.414 | 61.53                   |
| 15  | STM15       | 0.523                            | 0.482              | 0.424               | 0.374               | 0.301               | 0.421 | 57.62                   |
| 16  | STM16       | 0.516                            | 0.476              | 0.419               | 0.369               | 0.293               | 0.415 | 56.78                   |
| 17  | STM17       | 0.541                            | 0.448              | 0.395               | 0.350               | 0.289               | 0.404 | 53.51                   |
| 18  | STM18       | 0.554                            | 0.449              | 0.403               | 0.357               | 0.276               | 0.408 | 49.73                   |
| 19  | STM19       | 0.547                            | 0.508              | 0.460               | 0.433               | 0.367               | 0.463 | 67.05                   |
| 20  | STM20       | 0.569                            | 0.511              | 0.460               | 0.416               | 0.354               | 0.462 | 62.21                   |
| 21  | STM21       | 0.575                            | 0.541              | 0.522               | 0.502               | 0.465               | 0.521 | 80.81                   |
| 22  | STM22       | 0.538                            | 0.495              | 0.460               | 0.436               | 0.405               | 0.467 | 75.34                   |
| 23  | STM23       | 0.549                            | 0.510              | 0.454               | 0.417               | 0.357               | 0.457 | 65.07                   |
| 24  | STM24       | 0.550                            | 0.511              | 0.451               | 0.404               | 0.327               | 0.449 | 59.36                   |
|     | <b>Mean</b> | 0.535                            | 0.496              | 0.448               | 0.412               | 0.351               | 0.448 | 65.63                   |
|     |             | <i>LSD<sub>0.05</sub>: 0.067</i> |                    |                     | <i>CV%: 9.30%</i>   |                     |       |                         |

more salt tolerant than other crosses (Table 3). Research on salt tolerance in maize from Muhammad, et al. (2010) found that salt tolerances in maize varieties are very different, at which have high dry matter production and the ability for better growth and development in salt stress [10].

### Ion Na<sup>+</sup>, K<sup>+</sup> accumulation

As observed through experiments, the crosses of tolerant and saline sensitive seedlings were selected for measuring ion Na<sup>+</sup> and K<sup>+</sup> accumulation. The results in Table 4 show the levels of Na<sup>+</sup> accumulation in plants that increased from increasing the salt concentration, and the average Na<sup>+</sup> content in S0 was 0.375%, increased to 4.951% in S4 treatment. Among the crosses, STM18 accumulated the highest Na<sup>+</sup> at 5.552%, while STM21 showed the lowest (4.392%) in the same salt concentration of 200 mM NaCl.

**Table 4. The effect of salinity on ion Na<sup>+</sup> accumulation of different maize crosses.**

| No.         | Crosses | Na <sup>+</sup> content (% dry weight) |                       |                        |                        |                        | Mean  |
|-------------|---------|--|-----------------------|------------------------|------------------------|------------------------|-------|
|             |         | Treatments                             |                       |                        |                        |                        |       |
|             |         | S0<br>(0 mM<br>NaCl)                   | S1<br>(50 mM<br>NaCl) | S2<br>(100 mM<br>NaCl) | S3<br>(150 mM<br>NaCl) | S4<br>(200 mM<br>NaCl) |       |
| 1           | STM10   | 0.363                                  | 0.795                 | 1.733                  | 3.355                  | 4.509                  | 2.151 |
| 2           | STM13   | 0.373                                  | 0.848                 | 1.786                  | 3.408                  | 4.988                  | 2.281 |
| 3           | STM17   | 0.390                                  | 1.022                 | 2.579                  | 4.352                  | 5.472                  | 2.763 |
| 4           | STM18   | 0.363                                  | 1.102                 | 2.659                  | 4.432                  | 5.552                  | 2.822 |
| 5           | STM21   | 0.380                                  | 0.702                 | 1.639                  | 3.262                  | 4.392                  | 2.075 |
| 6           | STM22   | 0.380                                  | 0.943                 | 2.043                  | 3.665                  | 4.795                  | 2.365 |
| <b>Mean</b> |         | 0.375                                  | 0.902                 | 2.073                  | 3.746                  | 4.951                  | 2.409 |
|             |         | LSD <sub>0.05</sub> : 0.146            |                       | CV%: 13.40%            |                        |                        |       |

For potassium, the accumulation of K<sup>+</sup> in all crosses as shown to decrease with increasing salt concentrations (Table 5). The average ion K<sup>+</sup> accumulated in the crosses of S0 (0 mM NaCl) at 3.016%, which reduced to 1.931% in the S4 concentration (200 mM NaCl). The STM21 maintained K<sup>+</sup> absorption at the highest of all treatments, followed by STM10. The STM18 was recognized as the cross which showed the lowest K<sup>+</sup> uptake.

**Table 5. The effect of salinity on ion K<sup>+</sup> accumulation of different maize crosses.**

| No.         | Crosses | K <sup>+</sup> content (% of dry weight) |                       |                        |                        |                        | Mean  |
|-------------|---------|--|-----------------------|------------------------|------------------------|------------------------|-------|
|             |         | Treatments                               |                       |                        |                        |                        |       |
|             |         | S0<br>(0 mM<br>NaCl)                     | S1<br>(50 mM<br>NaCl) | S2<br>(100 mM<br>NaCl) | S3<br>(150 mM<br>NaCl) | S4<br>(200 mM<br>NaCl) |       |
| 1           | STM10   | 3.037                                    | 2.927                 | 2.787                  | 2.473                  | 2.183                  | 2.681 |
| 2           | STM13   | 3.027                                    | 2.933                 | 2.807                  | 2.517                  | 2.227                  | 2.702 |
| 3           | STM17   | 3.003                                    | 2.730                 | 2.220                  | 1.780                  | 1.480                  | 2.243 |
| 4           | STM18   | 2.977                                    | 2.733                 | 2.223                  | 1.693                  | 1.393                  | 2.204 |
| 5           | STM21   | 3.023                                    | 3.080                 | 2.860                  | 2.570                  | 2.280                  | 2.763 |
| 6           | STM22   | 3.030                                    | 2.937                 | 2.587                  | 2.312                  | 2.022                  | 2.577 |
| <b>Mean</b> |         | 3.016                                    | 2.890                 | 2.581                  | 2.224                  | 1.931                  | 2.528 |
|             |         | LSD <sub>0.05</sub> : 0.113              |                       | CV%: 12.10%            |                        |                        |       |

### Conclusions

Based on the results of these studies of saline tolerant maize cross combinations at the seedling stage, the conclusions are as follows:

1) Shoot length, root length, and plant dry weight of all crosses were reduced as salt concentration increased. The STM17, STM18 reduced the highest, while the STM21 presented the lowest reduction of both shoot length and root length. The STM21 produced the best salt tolerant index, followed by STM13 and STM10.

2) The accumulation of ion K<sup>+</sup> decreased, and the

absorption of Na<sup>+</sup> increased in the presence of salt stress. The STM21 showed the best accumulation of K<sup>+</sup> and elimination of Na<sup>+</sup>.

3) The effect of salinity on the crosses was very different, especially among them identified as STM21 and STM10, which had the highest salt tolerant index.

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