Screening, Inheritance and Linkage Marker Analyses of Salt Tolerance in Mutated Scented Japonica Rice (Oryza sativa L.)

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Abstract: We grew 1005 mutated scented japonica rice lines to the seventh steady genetic generation, and treated the seedlings with six true leaves with 300 mM NaCl for three days. Only the salt-tolerant line, SM61, survived. We obtained F1, F2 and F3 populations from the cross between SM61 and a salt-susceptible indica variety, TCS17. After culture with 200 mM NaCl for five days, SM61 and F1 (SM61 × TCS17; TCS17 × SM61) plants survived (R) while TCS17 plants did not (S). The R to S ratio in 513 F2 plants showed a good fit to the Mendelian 3 : 1 segregation ratio by a Chi-square test indicating that the salt-tolerance of SM61 was governed by a single dominant gene. The mutated salt-tolerance gene explained close to 100% of the total phenotypic variation, and was tightly linked to RM223 (marker) located on chromosome 8, which was different from the results of previous studies investigating the relationship of QTLs with salt tolerance. This is the first report of mapping tightly linked markers of a single dominant mutated salt-tolerance gene.

Key words: Inheritance, Mutated, Oryza sativa, Quantitative trait loci (QTLs), Salt tolerance, Scented, Seedling stage.

Rice (Oryza sativa L.) is a staple food and energy source for over half of the world’s population and is considered to be more salt-sensitive than other crops. Agricultural practices are often to blame for the incursion of salt water that causes the salinization of arable land (Rengasamy, 2006). The accumulation of salt in the soil reduces the growth and yield ability of rice (Hasegawa et al., 2000; Flowers, 2004). To improve the growth and yield, we need to develop plants with tolerance to salinity in the soil. Evaluating tolerance to saline conditions in plants requires evaluation of a complex set of physiological traits measured by components such as: plant survival scores, plant vigor, green leaf area, ion concentration and osmosis (Yeo et al., 1990; Sabouri and Sabouri, 2008). The main mechanisms of salt tolerance in crops include: ion homeostasis, osmotic homeostasis, and control and repair of oxidative stress damage (Verslues et al., 2006).

The primary objective of rice breeding programs is to develop elite salt-tolerant varieties suitable for salinized areas. It is difficult to introduce the salt-tolerance of indica varieties, Pokkali, Nona Bokra, and CSR10, into japonica or Basmati varieties that have superfine grain qualities and the high yield by conventional breeding methods. Breeding methods have been limited by the lack of identification of salt-tolerant japonica varieties (Lee, 1995), lack of studies on the mechanism of genetic control of salt tolerance (Lin et al., 2004), and a lack of linking between salt-tolerance traits and desirable agronomic characters (Akbah et al., 1985; Gregorio and Senadhira, 1993; Singh et al., 2000; Kaushik et al., 2003). QTL map studies on salt-tolerance provide information towards the improvement of plant breeding under saline conditions. In previous studies (Zhang et al., 1995; Lin et al., 1998; Gong et al., 1999; Prasad et al., 2000; Koyama et al., 2001; Lin et al., 2004; Ren et al., 2005; Lee et al., 2007; Sabouri and Sabouri, 2008), the location of QTLs for salt tolerance in rice was examined by using molecular markers. Zhang et al. (1995), Lin et al. (1998), Gong et al. (1999) and Prasad et al. (2000) found QTLs involved in salt tolerance of rice on chromosome 7, 5, 1 and 6. Ten QTLs for Na⁺ and K⁺ uptake, Na⁺ and K⁺ concentration, and Na⁺/K⁺ ratio in the shoot have been identified (Koyama et al., 2001). Lin et al. (2004) detected eleven QTLs that define the survival period of seedlings, as well as the traits of the shoots and roots in relation to salt tolerance in rice under salt stress on chromosomes 1, 4, 6, 7 and 9. Lee et al. (2007) mapped two salt tolerance QTLs at the young seedling stage on chromosomes 1 and 3. Sabouri and Sabouri (2008) found nine QTLs for chlorophyll content, root length, root dry weight and ion exchange on chromosomes 1, 3, 4, 7, 9 and 10, which increased salt tolerance at the seedling stage. Most QTLs related to salt-tolerance were located in a similar region on chromosome 1 (Gong et al., 1999; Koyama et al., 2001; Lin et al., 2004; Ren et al., 2005). Identification of new QTLs related to salt tolerance through marker-assisted selection (MAS) should be useful.
in rice breeding programs (Kaushik et al., 2003; Lin et al., 2004; Lee et al., 2007).

The purpose of this study was to analyze heritage characters and linkage markers of the screened line, SM61, for a mutated salt-tolerance gene by using F1, F2 and F3 populations derived from a cross between a salt-tolerant japonica line, SM61, and a salt-susceptible indica variety, TCS17. The results will help elucidate the mechanism controlling the salt-tolerance gene and will be useful in breeding programs of salt tolerance in rice.

Materials and Methods

1. Plant materials

The wild-type japonica cultivar, TNG67 (Huang, 1979), was treated with a chemical mutagen, NaN3, to produce the steady genetic line of mutated scented rice, CNY911303. Then, CNY911303 was treated with EMS to produce the second generation of 1005 mutated lines that developed to the sixth generation with superfine agronomic traits in the first season of 2006. These 1005 lines, at the three true leaf growth stage, growing in the National Chung Hsing University glasshouse were treated with 150 mM NaCl for three weeks. According to the procedure of Verma et al. (2007), 11 salt-tolerant lines were selected, grown and developed to the seventh generation. All 13 lines, including the 11 selected lines, CNY911303, and TNG67 seedlings with six true leaves, were treated with 300 mM NaCl for three days after (Ohta et al., 2002). SM61 was the only surviving line which produced seeds. This salt-tolerant japonica line, SM61, was hybridized with a salt-susceptible indica variety, TCS17, and F1 seeds were produced in the second season of 2007. The F1 plants were self-fertilized to develop the F2 seeds in the first season of 2008, and F2 plants produced F3 seeds in the second season of 2008.

2. Comparison of salt-tolerant lines

In order to better understand the degree of salt tolerance and differences of SM61 from the traditional salt-tolerant indica varieties, Pokkali, and Nona Bokra, these varieties were germinated on vermiculite and grown to the three true leaf stage. They were then transferred to hydroponic culture medium (modified Yoshida et al., 1976), and cultured in a net-house at the Chaiyi Agricultural Research Institute at a mean temperature of 30/20°C (day/night). Once every week the hydroponic culture medium was renewed and the pH was maintained at 4.7 to 4.8. SM61, Pokkali, and Nona Bokra at the six true leaf stage were cultured in hydroponic culture medium supplemented with 150, 200 or 250 mM NaCl for three days and then transferred to a hydroponic culture medium without NaCl. Each container (45 × 33.5 × 11.5 cm) held four SM61, Pokkali, and Nona Bokra plants. The experiment was performed with two replications.

3. Physiological analysis of salt tolerance

To examine the Na+, K+, and Cl- concentrations in the shoots and the roots and osmotic regulating ability of SM61, we performed the following two experiments. SM61 and CNY911303 at the six true leaf stage were cultured in hydroponic culture medium supplemented with 0 and 150 mM NaCl for one week, in a net-house at Chaiyi Agricultural Research Institute at a mean temperature of 30/20°C (day/night). Each container (45 × 33.5 × 11.5 cm) held six each of SM61 and CNY911303 plants. The experiment was performed with two replications. The shoots and roots of SM61 and CNY911303 were harvested and packaged in paper bags. The samples were dried at 100°C for 4 h, at 80°C for 3 days, and weighed. The Na+, K+, and Cl- were extracted from the dry shoots and roots and their concentrations were determined by the ICP and IC.

Second, SM61 and TNG67 plants with six true leaves were grown in the hydroponic culture medium containing 25% PEG for five days. Each container (45 × 33.5 × 11.5 cm) held four each of SM61 and TNG67 plants. The experiment was performed with two replications. Leaf rolling ratio (rolling leaves/total leaves) of SM61 and TNG67 plants was calculated. The means and standard errors were calculated using Microsoft Excel 2000 software.

4. Inheritance analysis of salt tolerance

Plants with six true leaves were grown in the hydroponic culture medium supplemented with 200, 250 or 300 mM NaCl for five days, in a net-house at Chaiyi Agricultural Research Institute at a mean temperature of 30/20°C (day/night). In each container (45 × 33.5 × 11.5 cm), six plants each of SM61 and TCS17 were planted. The experiment was performed with two replications.

To confirm that the gene controlling salt-tolerance in SM61 was a single dominant gene, we performed two experiments. First, SM61, TCS17, and F1 of the SM61 × TCS17 cross and reciprocal at the six true leaf stage, were grown in a hydroponic culture medium supplemented with 200 mM NaCl for five days and then transferred to a hydroponic culture medium without NaCl. In each container (45 × 33.5 × 11.5 cm), four plants each of SM61, TCS17 and F1 (SM61 × TCS17; TCS17 × SM61) were planted. The experiment was performed with two replications. F2 plants (SM61 × TCS17), 513 plants in total, were composed of 171 plants each of the three hybridized crosses (A1, A2 and A3) (60 × 48 × 16 cm container). SM61 was salt-tolerant while TCS17 and TNG67 were salt-sensitive. After the five-day culture, the 513 F2 plants were transferred to the hydroponic culture medium without NaCl. After two weeks, their salt-tolerant surviving (R) and salt-sensitive non-surviving (S) frequency distribution was calculated. The R to S ratio of F2 A1/A1, F2 A2/A2, F2 A1/A2, and F2 A1A1A2A2 populations was analyzed to fit a Mendelian 3 : 1 segregation ratio using a Chi-square test.
from SAS software. F_{A1}, F_{A2}, F_{A3}, and F_{A1+A2+A3} represent the 171 F_{2} plants derived from the A1, A2 and A3 crosses and the 513 F_{2} plants derived from the three hybridized crosses (A1+ A2+ A3).

5. Linkage analysis of salt-tolerant plants

As in the above experiments on inheritance, 290 F_{2} plants were cultured in a hydroponic culture medium in a plastic tray (60 × 48 × 16 cm) under non-shaded conditions at NaCl concentrations of 35/25ºC (day/night). At the four true leaf stage, the F_{2} plants were cut at about the 5 cm leaf level and DNA was extracted. The plants with six true leaves were grown in a hydroponic culture medium supplemented with 200 mM NaCl for five days and 52 F_{2} salt-tolerant surviving plants were selected. SM61 was salt tolerant while TCS17 and TNG67 were salt susceptible. Among the 52 F_{2} salt-tolerant surviving plants, 46 plants were screened for constructing the linkage markers map of the salt-tolerant mutated gene in SM61. After five days of culture, the plants were transferred to a hydroponic culture medium without NaCl. After one week, the 52 F_{2} salt-tolerant plants were transplanted to the field and the salt-tolerant surviving plants developed into F_{3} lines.

The F_{2} plants (10-31 plants in each line) were cultured in a hydroponic culture medium in a net-house at Chiayi Agricultural Research Institute during March and April in 2009, at a mean temperature of 30/20ºC (day/night). The F_{2} plants were cut at about the 5 cm leaf level and DNA was extracted. The plants with six true leaves were grown in a hydroponic culture medium supplemented with 200 mM NaCl for five days and 52 F_{2} salt-tolerant surviving plants were selected. SM61 was salt tolerant while TCS17 and TNG67 were salt susceptible. Among the 52 F_{2} salt-tolerant surviving plants, 46 plants were screened for constructing the linkage markers map of the salt-tolerant mutated gene in SM61. After five days of culture, the plants were transferred to a hydroponic culture medium without NaCl. After one week, the 52 F_{2} salt-tolerant plants were transplanted to the field and the salt-tolerant surviving plants developed into F_{3} lines.

The F_{3} plants (10-31 plants in each line) were cultured in a hydroponic culture medium in a net-house at Chiayi Agricultural Research Institute during March and April in 2009, at a mean temperature of 30/20ºC (day/night). The F_{3} plants were cut at about the 5 cm leaf level and DNA was extracted. The plants with six true leaves were grown in a hydroponic culture medium supplemented with 200 mM NaCl for five days and 52 F_{2} salt-tolerant surviving plants were selected. SM61 was salt tolerant while TCS17 and TNG67 were salt susceptible. Among the 52 F_{2} salt-tolerant surviving plants, 46 plants were screened for constructing the linkage markers map of the salt-tolerant mutated gene in SM61. After five days of culture, the plants were transferred to a hydroponic culture medium without NaCl. After one week, the 52 F_{2} salt-tolerant plants were transplanted to the field and the salt-tolerant surviving plants developed into F_{3} lines.

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Fig. 1. Salt-tolerance of the wild-type cultivar and the mutated scented japonica plants. The wild-type cultivar (TNG67), the mutated parent variety (CNY911303) and the mutant line (SM61) at the six true-leaf stage (lane 1) were cultured in hydroponic culture medium supplemented with 300 mM NaCl for three days (lane 2), and then transferred to the hydroponic culture medium without NaCl and grown for two weeks (lane 3). Each container had 12 (6×2) plants.

Fig. 2. Salt-tolerance of the traditional salt-tolerant indica varieties, Nona Bokra and Pokkali, and the mutant line, SM61. Plants at the six true-leaf stage (lane 1) were cultured in hydroponic culture medium supplemented with 150, 200 or 250 mM NaCl for three days and then transferred to the hydroponic culture medium without NaCl and grown for 1 week (lane 2) or 2 weeks (lane 3). Each container had 12 (4×3) plants, 4 (4×1) plants from each line with two replications.

was examined. The results showed that leaf-injury level of SM61 was lower than that of TCS17 and that SM61 was more salt-tolerant than TCS17. Among the three different concentrations, 200 mM NaCl was the best for comparison of performance and appearance between SM61 and TCS17 (Fig. 3). The results showed that SM61 was salt-tolerant, and TCS17 salt-sensitive. Thus, we treated the F1 and F2 plants derived from SM61 and TCS17 with 200 mM NaCl to study the heritage traits of the mutated salt-tolerance gene in SM61 on the basis of their salt-tolerant performance.

F1 and F2 populations were derived from a cross between SM61 and TCS17. At the six true leaf stage, SM61, TCS17, F1 (SM61×TCS17; TCS17×SM61) and F2 rice seedlings were treated with 200 mM NaCl for five days and were then transferred to a hydroponic culture medium without
| Treatment | 1 | 2 | 3 |
|-----------|---|---|---|
| 200 mM NaCl | ![Image](SM61 SM17) | ![Image](SM61 SM17) | ![Image](SM61 SM17) |
| 250 mM NaCl | ![Image](SM61 SM17) | ![Image](SM61 SM17) | ![Image](SM61 SM17) |
| 300 mM NaCl | ![Image](SM61 SM17) | ![Image](SM61 SM17) | ![Image](SM61 SM17) |
| TCG17 (left) and TCS17 (right) | ![Image](SM61 SM17) | ![Image](SM61 SM17) | ![Image](SM61 SM17) |

Fig. 3. Salt-tolerance of the mutant line, SM61, and the other cross parent variety (traditional indica variety), TCS17. Plants at the six true-leaf stage (lane 1) were cultured in hydroponic culture medium supplemented with 200, 250 or 300 mM NaCl for three days (lane 2) or five days (lane 3). Each container had 12 (6 × 2) plants, 6 (6 × 1) plants from each line with two replications.

Fig. 4. Salt-tolerance of the salt-tolerant japonica parent line, SM61, the salt-susceptible indica parent variety, TCS17, and F1 of the SM61 × TCS17 cross and reciprocal plants. Plants at the six true-leaf stage (lane 1) were cultured in a hydroponic culture medium supplemented with 200 mM NaCl for five days and then transferred to a hydroponic culture medium without NaCl and grown for 4 days (lane 2) or 17 days (lane 3). Each container had 16 (4 × 4) plants, 4 (4 × 1) plants from each line with two replications.

Fig. 5. The salt-tolerant and salt-sensitive seedlings of the F1 plants derived from a cross between the salt-tolerant japonica line, SM61, and the salt-sensitive indica variety, TCS17. F1 plants at the six true-leaf stage were cultured in hydroponic culture medium supplemented with 200 mM NaCl for five days (a) and then transferred to a hydroponic culture medium without NaCl and grown for 1 week (b).
After five days of culture, the leaf-injury level was much lower in SM61 and F1 (SM61 × TCS17; TCS17 × SM61) than in TCS17. After the plants were transferred to the culture medium without NaCl for two weeks, SM61 and F1 (SM61 × TCS17; TCS17 × SM61) plants were survivals (R) while TCS17 plants were non-survivals (S) (Fig. 4, Table 3). The F1 line resembled SM61, indicating that salt tolerance at the seedling stage was dominant. Among 513 F2 plants grown in the culture medium without NaCl for two weeks, the number of R and S plants were 387 and 126, respectively. The R to S ratios of the F2-A1, F2-A2, F2-A3, and F2-A1+A2+A3 populations fitted a Mendelian 3 : 1 segregation ratio by the Chi-square test (Table 3). This suggests that the salt-tolerance of SM61 was governed by a single dominant gene.

4. Evaluation of salt tolerance in F2 and F3 linkage analyses

SM61 and TCS17 were treated with 200, 250 and 300 mM NaCl for five days. The results showed that SM61 was more salt-tolerant than TCS17 and that 200 mM NaCl was the best concentration to observe differences in leaf-injury between SM61 and TCS17. Second, since SM61 is a salt-tolerant japonica line and TCS17 is a salt-sensitive indica variety, they were expected to produce more polymorphic markers. Therefore the F1 plants derived from SM61 and TCS17 were treated with 200 mM NaCl to find the linkage relation between the polymorphic-marker genotypes and salt-tolerant phenotypes.

Of the 290 F2 (SM61 × TCS17) plants treated with 200 mM NaCl for five days 52 salt-tolerant plants survived and grew better than the 238 salt-sensitive plants (Fig. 5 a). Among the F2 plants transferred to the culture medium without NaCl and cultured for one week, the 52 salt-tolerant plants developed new leaves and roots quickly while the salt-sensitive plants did not survive (Fig. 5 b). We screened 46 of the 52 F2 salt-tolerant surviving plants to map the salt-tolerant mutated gene in SM61. These 52 salt-tolerant plants were grown in the field and 31 F2 plants survived and produced seeds. Twenty-nine of the 31 F2 plants were included in the 46 selected F2 plants. The same treatment was applied to the F3 lines (10-31 plants of each line), SM61, TCS17 and TNG67. SM61 plants were salt-tolerant (R) while TCS17 and TNG67 plants were salt-susceptible (S). The percentage of R in the F3 lines reached 87–100% which agrees with the segregated value of ≥75%. The R and S values in the 31 F3 lines therefore conform to non-segregation (Table 4).

**Table 1. Comparison of Cl, Na+, K concentration and Na+/K ratio in dry shoots and roots of the mutant line, SM61 and the mutated parent variety, CNY911303.**

| Treatment NaCl (mM) | Ion concentration (mg/g-1 dw) | Shoot | Root |
|---------------------|-------------------------------|-------|------|
|                     | Cl                            | SM61  | CNY911303 | SM61  | CNY911303 |
| 0                   | 9.20 ± 0.64 (100)             | 8.83 ± 0.31 (96) | 5.38 ± 0.80 (100) | 7.62 ± 0.19 (142) |
| 150                 | 42.90 ± 1.62 (100)            | 63.00 ± 6.58 (147)* | 34.20 ± 3.29 (100) | 49.80 ± 6.52 (146) |
| Na+                 | 0.97 ± 0.03 (100)             | 1.13 ± 0.03 (116) | 5.14 ± 0.91 (100) | 5.76 ± 0.19 (112) |
| 150                 | 31.15 ± 2.00 (100)            | 45.15 ± 4.76 (145) | 34.30 ± 1.56 (100) | 41.05 ± 0.95 (120)* |
| K                   | 31.90 ± 0.29 (100)            | 36.65 ± 0.49 (115) | 8.28 ± 0.92 (100) | 15.65 ± 1.18 (189) |
| 150                 | 22.20 ± 0.52 (100)            | 24.50 ± 0.81 (110) | 7.32 ± 0.33 (100) | 6.55 ± 0.15 (89) |
| Na+/K               | 0.03 ± 0.00 (100)             | 0.03 ± 0.00 (100) | 0.64 ± 0.04 (100) | 0.37 ± 0.02 (61) |
| 150                 | 1.40 ± 0.06 (100)             | 1.83 ± 0.13 (131)* | 4.69 ± 0.00 (100) | 6.27 ± 0.00 (134)** |

Means ± S.E. represents the averages of two samples, every sample has 6 rice plants.

Numbers in parentheses are the percentages of parent rice plants (CNY911303) compared to control rice plants (SM61) (represented by 100).

As compared to SM61, * and **, represent significant at P < 0.05 and 0.01 in CNY911303, respectively.

**Means ± S.E. represents the averages of eight plants for leaves rolling ratio.**

As compared to SM61, * and ***, represent significant at P < 0.05 and 0.01 in TNG67, respectively.

| Leaves rolling ratio | SM61   | TNG67 |
|----------------------|--------|-------|
| The third day        | 0.25 ± 0.06 | 0.76 ± 0.09** |
| The fourth day       | 0.28 ± 0.05 | 0.78 ± 0.07** |
| The fifth day        | 0.36 ± 0.05 | 0.83 ± 0.07** |

**Table 2. Leaf rolling ratio (rolling leaves/total leaves) of the mutant line, SM61, and the wild-type cultivar, TNG67.** The rice plants at the six true-leaf stage were cultured in hydroponic culture medium containing 25% PEG for five days.

| Leaves rolling ratio | SM61 | TNG67 |
|----------------------|------|-------|
| The third day        | 0.25 ± 0.06 | 0.76 ± 0.09** |
| The fourth day       | 0.28 ± 0.05 | 0.78 ± 0.07** |
| The fifth day        | 0.36 ± 0.05 | 0.83 ± 0.07** |

Means ± S.E. represents the averages of eight plants for leaves rolling ratio.

As compared to SM61, * and ***, represent significant at P < 0.05 and 0.01 in TNG67, respectively.
These results revealed that the salt-tolerance of the 31 F3 plants was governed by a homozygote dominant allele.

5. **Linkage analysis of a salt-tolerance gene**

Since the salt-tolerance of SM61 was governed by a single dominant gene, we needed to use salt-sensitive non-survivals to map the linkage markers of the salt-tolerance gene in SM61. However, the screened environment under non-shaded conditions at National Taiwan University during July and August produced a majority of dead plants preventing the selection of salt-sensitive non-survivals for mapping. Moreover, the SM61 and TCS17 cross had the

| Rice population | Observed frequency | X² | P |
|-----------------|-------------------|----|---|
| SM61           | 8                 | 0  | 8 |
| TCS17          | 0                 | 8  | 8 |
| SM61 x TCS17 (F₁) | 8             | 0  | 8 |
| TCS17 x SM61 (F₁) | 8              | 0  | 8 |
| F₂, a₁         | 122               | 49 | 171 | 1.2183 | 0.2697 |
| F₂, a₂         | 138               | 33 | 171 | 2.9649 | 0.0851 |
| F₂, a₃         | 127               | 44 | 171 | 0.0487 | 0.8253 |
| F₂, A₁+A₂+A₃   | 387               | 126| 513 | 0.0526 | 0.8185 |

1) Salt-tolerant survival rice plants.  
2) Salt-sensitive non-survival rice plants.  
3) 171 F₂ rice plants derived from A₁ cross (SM61 x TCS17).  
4) 171 F₂ rice plants derived from A₂ cross (SM61 x TCS17).  
5) 171 F₂ rice plants derived from A₃ cross (SM61 x TCS17).  
6) 513 F₂ rice plants derived from three hybridized crosses (A₁+ A₂+ A₃).

| Line | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|------|---|---|---|---|---|---|---|---|---|----|
| F₂   | 21 | 20 | 12 | 11 | 16 | 18 | 20 | 26 | 26 | 22 |
| S%   | 100.00 | 95.24 | 92.31 | 100.00 | 100.00 | 94.74 | 86.96 | 96.30 | 100.00 | 100.00 |
| S%   | 0.00 | 4.76 | 7.69 | 0.00 | 0.00 | 5.26 | 13.04 | 3.70 | 0.00 | 0.00 |

| Line | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|------|---|---|---|---|---|---|---|---|---|----|
| F₂   | 30 | 18 | 23 | 12 | 23 | 13 | 18 | 13 | 14 | 9 |
| S%   | 100.00 | 100.00 | 100.00 | 100.00 | 88.46 | 100.00 | 100.00 | 93.33 | 90.00 |
| S%   | 96.77 | 7.69 | 0.00 | 11.54 | 0.00 | 0.00 | 0.00 | 0.00 |

| Line | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|------|---|---|---|---|---|---|---|---|---|---|----|
| F₂   | 31 | 26 | 15 | 22 | 26 | 27 | 25 | 28 | 27 | 23 | 29 |
| S%   | 100.00 | 100.00 | 100.00 | 100.00 | 96.30 | 96.43 | 100.00 | 100.00 | 100.00 |
| S%   | 0.00 | 0.00 | 0.00 | 0.00 | 3.70 | 3.37 | 0.00 | 0.00 |

1) The 31 F₂ lines derived from the salt-tolerant surviving F₁.  
2) Salt-tolerant survival rice plants.  
3) Salt-sensitive non-survival rice plants.  
4) The percentage of salt-tolerant survival rice plants.  
5) The percentage of salt-sensitive non-survival rice plants.
japonica distortion segregation problem. Therefore, we used 46 salt-tolerant plants described in Materials and Methods to perform the experiment.

The genotypes of TNG67, TCS17, heterozygote, and non-detection are represented by A, B, H, and N, respectively.

Table 5. The genotypes of markers in 46 salt-tolerant F2 plants on chromosome 1−12 and in 31 salt-tolerant F2 plants on chromosome 8 (bold letters).

| Chr | Location (cM) | Marker | A | H | B | N |
|-----|---------------|--------|---|---|---|---|
| 1   | 38.8          | RM259  | 9 | 25| 12| 0 |
| 1   | 55.7          | STS326 | 9 | 28| 9 | 0 |
| 1   | 86.0          | RM5638 | 8 | 28| 10| 0 |
| 1   | 95.7          | S756   | 8 | 27| 11| 0 |
| 1   | 129.0         | RM5411 | 10| 24| 12| 0 |
| 1   | 181.8         | RM6840 | 14| 21| 11| 0 |
| 2   | 43.4          | RM5356 | 4 | 25| 17| 0 |
| 2   | 62.2          | RM1038 | 9 | 25| 12| 0 |
| 2   | 71.3−77.8     | RM341  | 9 | 24| 13| 0 |
| 2   | 80.5          | RM475  | 2 | 24| 20| 0 |
| 2   | 138.0−140.9   | RM250  | 17| 18| 9 | 2 |
| 2   | 157.9         | RM555  | 13| 23| 10| 0 |
| 3   | 6.3           | RM22   | 11| 22| 13| 0 |
| 3   | 44.4−46.6     | RM251  | 7 | 23| 16| 0 |
| 3   | 55.8          | RM282  | 3 | 25| 17| 1 |
| 3   | 73.5−76.6     | C53358 | 4 | 24| 18| 0 |
| 3   | 91.1          | RM16   | 7 | 15| 20| 4 |
| 3   | 115.6         | SLS178 | 10| 15| 21| 0 |
| 3   | 140.1         | RM8267 | 8 | 21| 16| 1 |
| 4   | 8.7           | C52099 | 0 | 20| 26| 0 |
| 4   | 28.6          | SLS189 | 5 | 15| 25| 1 |
| 4   | 77.9−78.2     | RM252  | 16| 16| 14| 0 |
| 4   | 87.1−94.4     | RM303  | 12| 20| 14| 0 |
| 4   | 108.2         | S13714 | 10| 23| 13| 0 |
| 5   | 4.6           | RM3706 | 16| 21| 9 | 0 |
| 5   | 111.6         | RM480  | 11| 26| 9 | 0 |
| 6   | 6.0           | RM589  | 6 | 21| 16| 3 |
| 6   | 51.0          | SLS163 | 9 | 18| 16| 3 |
| 6   | 56.3          | RM527  | 2 | 20| 24| 0 |
| 6   | 100.8−103.0   | RM528  | 9 | 20| 15| 2 |
| 6   | 105.1         | RM530  | 3 | 22| 19| 2 |

The genotypes of TNG67, TCS17, heterozygote, and non-detection are represented by A, B, H, and N, respectively.
were 15, 12, and 4, respectively, and those of the SLS188 marker (92.2 cm) 9, 18, and 4, respectively. (Table 5). Based on the linkage correlation between the salt-tolerant marker genotypes and phenotypes, the phenotypic variance in the F$_2$ plants was explained by the RM223 marker on chromosome 8. Thus, the salt-tolerance gene, S78, which conferred the salt tolerance of SM61 was mapped on chromosome 8 flanked between RM331 and RM223 markers.

**Discussion**

In the SM61 × TCS17 cross and its reciprocal, the F$_1$ plants showed salt-tolerance, resembling SM61. Chi-square values indicated that the F$_2$ populations had good fits to a 3 : 1 segregation ratio for salt-tolerant (R) and salt-sensitive (S) traits. These results indicate that the salt-tolerance of SM61 was governed by a single dominant gene. These results differ from those obtained in some traditional indica salt-tolerant varieties, such as Pokkali, Nona Bokra, and Kararrata, in which inheritance of salt-tolerance is known to be governed by polygenes (Zhang et al., 1995; Lin et al., 1998; Gong et al., 1999; Prasad et al., 2000; Koyama et al., 2001; Lin et al., 2004). This is the first report that in F$_1$ and F$_2$ salt-tolerant rice plants, the salt-tolerance is governed by a single dominant gene. By analyzing the inheritance of the single dominant salt-tolerance gene in SM61, we can find the best method for analysis of the linkage markers of SM61 for the mutated salt-tolerance gene.

We selected 46 F$_2$ salt-tolerant plants to map the linkage markers of the mutated salt-tolerance gene, SM61. Thirty-one F$_2$ lines derived from the F$_1$ salt-tolerant plants were used to evaluate the genotypes of F$_2$ plants. We found that the SM61 mutated salt-tolerance gene, S78, is located on chromosome 8, different from those previous QTL regions previously discovered on chromosome 1, 3, 4, 5, 6, 7, 9, 10 and 12 (Zhang et al., 1995; Lin et al., 1998; Gong et al., 1999; Prasad et al., 2000; Koyama et al., 2001; Lin et al., 2004; Ren et al., 2005; Lee et al., 2007; Sabouri and Sabouri, 2008), but they did not refer to tightly linked markers for a single dominant salt-tolerance gene. In these reports, QTLs explained less than 50% of the total phenotypic variance for the salt-tolerance (Zhang et al., 1995; Lin et al., 1998; Gong et al., 1999; Prasad et al., 2000; Koyama et al., 2001; Lin et al., 2004; Ren et al., 2005; Lee et al., 2007; Sabouri and Sabouri, 2008), and most QTLs accounted for less than 30% of the salt-tolerance. Our study was therefore the first to map for tightly linked markers of a single dominant mutated salt-tolerance gene, explaining 100% of the phenotypic variation. These linkage markers can efficiently provide the fine mapping, cloning and sequence comparison, as well as the functions of the new mutated salt-tolerance gene. Results from such studies would answer the question of how the gene controls osmotic regulation and ionic absorbance. In order to understand the function of S78 we have begun a map-based cloning of the gene, using the 387 salt-tolerant surviving plants derived from the 513 F$_2$ plants.

**Acknowledgements**

We thank Dr. Yong-Pei Wu and Chaiyi Agricultural Research Institute for supporting materials, labs, and fields, and Dr. Yann-Rong Lin and the Laboratory of Genetics, Department of Agronomy, National Taiwan University for providing genetic analysis, labs, and friendly help. We also thank Dr. Wen-Shin Lin and the Laboratory of Biometry, Department of Agronomy, National Chung Hsing University for providing statistical analysis, and Mr. Woei-Shyuan Jwo and the Agricultural Research Institute for offering materials and experience.

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