Morphological, Chemical and Molecular Evaluations For Several Rice (Oryza Sativa L.) Races Grown Under Salt Stress Levels

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Abstract

A field experiment was carried out at the Agricultural Research and Experiments Station of the College of Agriculture, AL-Muthanna University during the summer season 2020. Pots of 15 cm diameter were used for planting the genotypes, with (SS, F-RCBD) and three replications. Ten different genotypes rice were used (FR1, FR2, FR15, FR16, FR17, FR18, FR21, FR25, FR27), as well as the rice varieties, which are Pokkali, Anber-33 and Jasmine, were irrigated by three levels of salty water (4.5, 7.5 and 15 ds/m). The results of the experiment showed that the tolerance of the genotypes FR21 and FR12 to salinity was associated with containing low concentrations of sodium (0.38 and 0.42%) respectively in the shoot, and a high concentration of potassium in the shoot (1.22 and 1.23%) respectively, and this result was reflected in the ratio of potassium to sodium in the shoot total was (3.30 and 2.95%) compared to the salt-tolerant variety Pokkali. Salinity caused a significant decrease in all the studied traits of the genotypes, especially at levels 7.5 and 15 ds/m, in growth traits (plant height, flag leaf area and panicle length), yield and its components (number of productive tillers, weight of 1000 grains and yield per plant). The most potent varieties in salt tolerance were FR12 and FR21, as they gave the highest yield of grains (11.72 and 16.67 g / plant, respectively), flag leave area (30.47 and 27.76 cm² respectively). Detection for OsHKT4 and OsHKT6 by PCR proved the presence of primary locus that utilized by each gene with successfully fused genes as well as their stability under Iraqi cultivation circumstances, particularly in the case of FR12 and FR21 where OsHKT4 lanes were very obvious (2840 Kb) and the bands of OsHKT6 at (2324 Kb), which resemble to that in Pokkali Japanese salt tolerance rice. These results suggested the variation among investigated rice varieties in their tolerance to salts, where FR12 and FR21 were best in their performance under applied the salt levels conditions.

Key words: Rice, Salt stress, Genotype, Physiological trait and molecular analysis.

1. Introduction

The rice crop (Oryza sativa L.) is one of the most important strategic crops in the world and a staple food for more than half of the world’s population, with a global production of 61.853 million tons, which is the highest among cereal crops, although it ranks second after wheat in terms of the cultivated area of 214 million hectares¹, more than 90% of rice is produced and consumed in Asia [1]. In Iraq, Rice comes after wheat and barley in terms of cultivated area and productivity. The average area of rice planted in Iraq in 2019 was about 464.37 hectares and produced approximately 574.710 tons of rice, with a productivity rate of 4.495 tons per hectares.[2], these are low production levels compared to rice-producing countries, which calls for attention to increase production using all available means one of the most important of these expansion in the cultivation of high-productivity genotypes and the lack of reliance on the variety Anber-33 on a large scale, which is the dominant variety in agriculture despite its low productivity. The problem of salinity is also one of the environmental determinants of rice production which is expanding, and the area of saline or salinity-affected lands will reach about 25% by 2050 especially in the delta coastal areas [3], which constitutes more than 65% of the global production area, that making salinity one of the factors which pose a threat to global food security. Rice has been classified as sensitive to salt stress [10], because of its direct and indirect effect on vegetative growth, yield and this effect depends on the growth stage and the duration of exposure to stress also, salinity of 6.9 dS.m⁻¹ is enough to reduce rice yield by 50% [4]. The mechanisms of salt stress tolerance in rice plants result from complex interactions and close interaction between environmental and genetic factors including preventing or reducing the absorption of sodium ions (Na⁺) through the roots and excluding it and expelling it to the old leaves or confining it within the gaps, salinity also has an effect on cell division and cell expansion of the growth areas in the plant, as well as reducing the growth of the vegetative system, reducing dry mass content, increasing shoot: root ratio, reducing leaf size, and thus decreasing grain production.
In order to reduce the problem of salt stress and reduce its harmful effects, it is to follow breeding and genetic improvement programs to produce improved compositions or varieties of salt-tolerant rice by identifying some genes that can contribute to the tolerance of salt stress in plants, especially in rice, by controlling the absorption of sodium or chloride ions. Regulating ion transport, protecting osmotic stress and accelerating growth [5].

This requires first understanding the basic molecular mechanisms of salinity tolerance in general, because salt stress tolerance is a quantitative trait that is controlled by a large number of genes, which contributed to the success of breeding resistant or salt-tolerant varieties, with understanding the contribution of these mechanisms at the cellular and molecular levels with regard to their functions under field conditions. It is still not fully understood, and the importance and role of genotypes being the basis for any production program in the field of ring crops and dealing with the problem of salinity and salinity. This experiment aim to determining which genotypes are more tolerant of salt stress through the role of some genes in controlling the absorption and distribution of sodium ions and the ionic balance of sodium with potassium in different plant tissues, in addition to knowing the effect of salt stress on some growth characteristics, yield and it is components.

2. Material and Methods

The experiment was carried out at the second agricultural research station of the College of Agriculture, Al-Muthanna University. Ten genetically modified genotype of rice (using CRISPR-Cas9 technology) [6], were planted, in addition to three varieties (Anber-33, Jasmine and Pokkali) which using as control, in the nursery on June 17, 2020, in seed trays, it was transferred on July 17, 2020 to the field and planted in pots of dimensions (50 cm high x 30 cm in diameter) with three pots per experimental unit, and each pot contained 3 plants. The plants were exposed to three salt concentrations (4.5, 7.5 and 15 ds.m⁻¹), which are represented by (S1, S2, and S3). A factorial experiment was carried out according to the split block design, with three replications, with 39 experimental units for each replicate the vertical distribution included salinity levels (4.5, 7.5 and 15 ds.m⁻¹) while the horizontal distribution included the genotypes. When signs of full maturity appear (yellow plants, yellow flag leaves and yellow panicle), the plants were harvested on 21 November, 2020. At the stage of 75%, flowering the plant height (cm) and flag leaf area (cm²), at maturity the number of productive tillers, panicle length, Wight of 1000 grain and grain yield per plant were recorded in 13 genotypes and the plants were harvested on 21 November 2020. The harmful effects induced by salinity were computed in percent reduction over control (ROC %) with the following formula.

\[
\text{Value in control – value in saline environment} \times 100
\]

\[
\text{Value in control}
\]

2.1 Determination of the concentration of Na⁺ and K⁺ in plants

The concentration of Na⁺ and K⁺ in the shoots was determined according to [7], the ratio of K⁺/Na⁺ in each sample of the shoot was estimated by dividing the K⁺ and Na⁺ results.

2.2 Molecular investigation with PCR technology

The molecular analyzes were carried out in the laboratories of the Biotechnology Research Center - Al-Nahrain University, collected clean leaves and free from disease and insect infestations for each genotype separately, which were used in the isolation and analysis of DNA using PCR technique to detect the presence of the gene sequences (OsHKT4 and OsHKT6) (Table 1). DNA was extracted from the leaves of young rice plants according to the Genomic DNA Mini Kit (Plant) method recommended by Gene aid Corporation.

| Genes Name  | Primer Name | Sequence (5' → 3') | TM   |
|------------|-------------|--------------------|------|
| OsHKT4     | Forward     | GAGGATCGGAGTCATCAGCTTTCG | 58.8 °C |
|            | Reverse     | TTCTCAACAAATTTTTTTGGGCCTTATAGAAG | 58 °C |
| OsHKT6     | Forward     | CAGCTCTGAAGTCTAAGACCGAGATGA | 58 °C |
|            | Reverse     | TTCTCAACTATTGTGGTAGCTAAACGC | 58 °C |

2.3 PCR polymerase chain reaction

The polymerase chain reaction (PCR) was test conducted using the My Genie 96/384 Thermal Block (Korean-made), after completing the extraction DNA and obtaining the DNA solution of the plants under study, the Forward and Reverse primers of the OsHKT4 and OsHKT6 genes were selected in this study as shown in Table 1) [1], which was supplied by the South Korean company BIONEER, the reaction materials protocol was applied by adding the ingredients in a tube for the PCR
machine, to multiply the extracted DNA according to the programming of the polymerase chain reaction device and its steps (37 cycles) for two hours according to [8].

2.4 Statistical analysis

After the data collection, it was classified, arranged and statistically analyzed according to the statistical program Genstat Discovery 4, and a comparison of the averages of the coefficients was made at the 0.05 probability levels.

3. Results and Discussion

The analysis of variance for different rice genotypes for quantitative traits are shown in (Table 2). Analysis of variance indicated that the difference among genotypes for all the traits under study, plant height (cm), Flag leave area (cm²), panicle length; no. of productive tillers (plant⁻¹), 1000-grain weight and yield per plant were highly significant for genotype and salinity. However, the interaction between genotype and salinity showed non-significant for plant height (cm), Flag leave area (cm²), panicle length and no. of productive tillers.

| So.v | d.f | Plant height | Flag leaf area | Panicle length | No. of productive tiller | 1000 grain weight | Grain yield per plant | K⁺ % | Na⁺% | K⁺/Na⁺ |
|------|-----|--------------|----------------|---------------|-------------------------|------------------|----------------------|-----|------|--------|
| R    | 2   | 62.72        | 8.42           | 12.93         | 0.54                    | 8.81             | 27.65                | 0.001 | 0.030 | 0.232  |
| G    | 12  | 422.60*      | 162.54*        | 36.23*        | 21.26*                  | 36.71*           | 205.72*              | 0.201* | 0.401* | 4.542* |
| E(a) | 24  | 21.34        | 21.49          | 1.96          | 1.08                    | 0.50             | 4.96                 | 0.001 | 0.005 | 0.016  |
| S    | 2   | 3359.23*     | 947.18*        | 203.70*       | 154.82*                 | 169.88*          | 1328.62*             | 3.901* | 2.805* | 32.57* |
| E(b) | 4   | 24.98        | 25.34          | 0.368         | 0.667                   | 1.04             | 5.14                 | 0.003 | 0.008 | 0.164  |
| (G×S)24 | 23.57 | 14.53       | 3.39           | 3.24*         | 1.76*                   | 9.76*            | 0.114*               | 0.083* | 0.291* |        |
| E (c) | 48  | 18.42        | 14.13          | 2.17          | 0.856                   | 0.46             | 3.50                 | 0.001 | 0.003 | 0.021  |
| Total | 116 |              |                |               |                         |                  |                      |      |      |        |

3.1 Plant height (cm)

The (Table 3) indicates the effect of salinity levels on plant height (cm) to the genotypes and their interaction. The varieties Anber-33 showed remarkable superiority and gave the highest average for this trait, which amounted to 83.59 cm, while the lowest average plant height were recorded for the genotype Jasmine, which reached 55.15 cm, while the genotypes showed closeness in their average plant height with non-significant difference among FR17, FR15, FR12, FR21 and FR25 genotypes. Salinity levels showed significant variation in plant height for performance of genotypes. The concentration S1 was significantly superior with plant height reached 80.59 cm compared to S2 and the S3 concentration, which gave averages of 70.60 and 62.05 cm, respectively, while the FR2 genotype recorded the lowest average for flag leaf area, which gave 16.72 cm² (Table 4), it is possible to attribute due to the superiority of the genotype to their relative superiority in plant height (Table 3), which results in the production of more dry matter due to the growth of cell size, the decrease in vital activities and damage to the chloroplasts with the lack and shortness of the internodes, which leads to a reduced in plant height. This result was confirmed [9], who indicated that the salinity increase from (6-12 dS.m⁻¹) led to a reduced in plant height of rice from (119.6 to 109.7 cm), with percent reduction over control 8.27%.

3.2 Flag leaf area (cm²)

The results was noticed that there was a significant increase in flag leaf area for the genotypes (FR21, FR17 and FR12), which gave the highest averages amounted to 30.47, 28.13 and 27.76 cm² respectively, while the FR2 genotype recorded the lowest average for flag leaf area, which gave 16.72 cm² (Table 4), it is possible to attribute due to the superiority of the genotype to their relative superiority in plant height (Table 3), which results in the production of more dry matter due to the length of the vegetative growth period and better light interception due to plant heights, which contributed to greater growth and expansion of the flag leaf, the results in same table also indicated that there were significant differences for the flag leaf area, which reduced with increasing levels of salinity, as the level S1 gave the highest average of the flag leaf area which gave 27.41 cm², outperforming that of the salinity level S2 which in turn outperformed the level S3 as they gave averages of 21.82 and 17.59 cm respectively, as percent reduction of 20.39% and 35.83% compared to level S1 respectively, that is may be due to attributed to the exposure to high concentrations of NaCl results in a decrease in plant growth and productivity, inhibition of leaf growth and a decrease in the rate of elongation due to the ionic stress caused by the excessive accumulation...
of toxic ions, including Na+ (Table 4), which caused premature aging and defoliation of adult leaves, thus reducing the leaf area available for light absorption.

### Table 3. Effect of genotypes and salinity levels and their interaction on plant height (cm)

| Genotype | Level of salinity | Average of genotype |
|----------|------------------|---------------------|
|          | S1               | S2                  | S3                  |
| FR1      | 80.22            | 71.55               | 61.78               |
| FR2      | 80.78            | 65.00               | 59.44               |
| FR12     | 85.50            | 75.66               | 63.55               |
| FR15     | 81.56            | 76.00               | 69.00               |
| FR16     | 73.89            | 62.56               | 53.22               |
| FR17     | 89.11            | 75.22               | 63.22               |
| FR18     | 78.22            | 67.22               | 57.78               |
| FR21     | 81.00            | 72.78               | 68.44               |
| FR25     | 82.44            | 72.56               | 65.22               |
| FR27     | 81.33            | 68.33               | 59.89               |
| Jasmine  | 58.56            | 55.55               | 51.33               |
| Anber -33| 95.00            | 84.89               | 70.89               |
| Pokkali  | 80.11            | 70.45               | 62.89               |
|          | 80.59            | 70.60               | 62.05               |

### Table 4. Effect of genotypes and salinity levels and their interaction on flag leaf area (cm²).

| Genotype | Level of salinity | Average of genotype |
|----------|------------------|---------------------|
|          | S1               | S2                  | S3                  |
| FR1      | 27.52            | 18.37               | 17.57               |
| FR2      | 20.59            | 15.97               | 13.59               |
| FR12     | 33.14            | 26.53               | 23.62               |
| FR15     | 25.51            | 20.16               | 19.13               |
| FR16     | 23.07            | 18.08               | 10.85               |
| FR17     | 35.14            | 28.05               | 21.21               |
| FR18     | 30.38            | 21.00               | 16.95               |
| FR21     | 39.10            | 28.45               | 23.87               |
| FR25     | 23.95            | 19.18               | 15.22               |
| FR27     | 20.97            | 20.70               | 18.31               |
| Jasmine  | 21.73            | 20.47               | 14.72               |
| Anber -33| 29.96            | 22.76               | 19.06               |
| Pokkali  | 25.33            | 23.97               | 14.58               |
|          | 27.41            | 21.82               | 17.59               |

### 3.3 Panicle length (cm)

The genotype FR12 gave the highest average of 24.25 cm, followed by genotypes Anber-33, FR1 and FR25, which averages of 21.89, 21.73 and 21.58 cm, respectively, whereas, the genotype FR16 gave the lowest average for panicle length, which gave 16.63 cm, which did not differ significantly from the varietiesJasmine, which gave an average of 17.43 cm (table 5), it is possible to explain the reason for the differences between the genotypes in the panicle length due to the action of the extra and non-extra genes, that is, by the action of the genes of dominance and superiority [10]. The results of the same table also showed a significant decrease of the panicle length with an increase in salinity levels, as the salinity level S1 gave the highest average of 22.43 cm, and the percentage of reduction for levels S2 and S3 was 10.10 and 20.38 %, respectively, the reason for the increase in the panicle length of the level S1 may be attributed to its superiority in plant height (Table 3) and the flag leaf area (Table 4) which together increased the rate of production of the representative materials, which led to reducing competition between parts the plant and within one plant, and that is reflected to increase the panicle length. These results was agreement with [11].
3.4 Number of productive tillers

The genotype FR12 gave the highest averages of Number of productive tillers per plant, which amounted to 13.45 panicle per plant, while the number of productive tillers decreased significantly in the FR17 genotype, which amounted to 7.71 tillers per plant, and the panicle per plant for the rest genotypes ranged between (8.07 - 10.65) tillers per plant (table 6). Perhaps the reason for the superiority of genotypes which recorded the highest average in the flag leaf area (Table 4), which led to an increase in the number of productive tillers due to the increase in the period of photosynthesis process and dry matter accumulation rates, which jointly pushed to increase, whereas the number of productive tillers decreased significantly with an increase in salinity levels starting from the salinity level of 7.5 dSm⁻¹, the salt level S1 gave the highest average of 11.43 tillers per plant compared with levels S2 and S3, which averaged 9.29 and 7.45 tillers per plant respectively, percentage reduction of 18.72 and 34.82% for levels S2 and S3 respectively, that is due to increase in salinity levels may be attributed to the failure of some branches to productive panicle due to the incomplete growth and development in a natural way, and due to the imbalance in the nutritional balance in the soil and the hormonal balance in the plant because effects of salinity, in addition, the superiority of the level S1 in flag leaf area (Table 4), which are the possible pillars of prolonging the photosynthesis period and increasing the production of dry matter, which ensured the natural development of tillers to become productive.

The results of the interaction between genotypes and salinity levels indicated significant differences, as the genotype FR12 gave the highest average which reached 15.06 tillers per plant which did not differ significantly from the genotype FR2 and Jasmine which averaged 14.28 and 14.22 tillers per plant respectively under S1 level of salinity, while the genotype FR12 showed a remarkable superiority, with their averages which amounted to 13.67 and 11.61 tillers per plant under the salt stress levels 7.5 and 15 dS.m⁻¹ respectively, with the percentage reduction which amounted to 9.23 and 22.91%, respectively, while the genotype FR17 had the lowest averages, which were 8.94 and 7.33 tillers per plant respectively under salt level 7.5 and 15 dS.m⁻¹ followed by genotype FR15 which recorded 4.94 tillers per plant under salt level 15 dS.m⁻¹, these results are in agreement with [12] which recorded a decrease in the number productive tillers of rice when the salinity levels increased from 6-12 dS.m⁻¹.

3.5 Wight of 1000 grain (g)

The trait of the weight of 1000 grains showed a significant difference between the genotypes, as the genotype FR17 gave the highest value of 21.28 (g), which did not differ significantly of the genotype FR21 with an average of 21.24 (g), while the variety Jasmine gave the lowest value of 13.83 (g) (Table 7). The salinity levels showed significant differences, as the increase in salinity led to a decrease in the weight of 1000 grains, as the control level of S1 salinity gave the highest average weight of 1000 grains of 19.88 g, while the reduction of percentage was by 9.05 and 20.92% for levels S2 and S3 respectively, The reason may be due to effect of salinity on grain weight is probably a reflection of the extent of root and leaf growth in plants that has to do with the ability of the source and sink to supply nutrients and water through the roots and its relation to the ability of the vegetative growth to effective on the photosynthesis [13].

| Genotype   | Level of salinity | Average of genotype |
|------------|-------------------|---------------------|
|            | S1    | S2    | S3    |                     |
| FR1        | 23.84 | 21.70 | 19.66 | 21.73               |
| FR2        | 21.72 | 20.52 | 16.52 | 19.59               |
| FR12       | 25.65 | 24.39 | 22.71 | 24.25               |
| FR15       | 23.30 | 20.20 | 18.69 | 20.73               |
| FR16       | 18.37 | 17.39 | 14.13 | 16.63               |
| FR17       | 21.60 | 19.69 | 17.64 | 19.64               |
| FR18       | 20.24 | 19.80 | 16.67 | 18.90               |
| FR21       | 22.24 | 21.22 | 17.11 | 20.19               |
| FR25       | 24.64 | 20.80 | 19.30 | 21.58               |
| FR27       | 23.78 | 19.76 | 17.83 | 20.46               |
| Jasmine    | 20.23 | 16.17 | 15.89 | 17.43               |
| Anber -33  | 22.45 | 22.11 | 21.10 | 21.89               |
| Pokkali    | 23.49 | 18.34 | 14.88 | 18.90               |
| Average of salinity level | 22.43 | 20.16 | 17.86 |
| L.S.D (0.05) | 1.36 | 0.38 | N.S. |
In case of interaction between the genotypes and salinity levels, the FR21 gave the highest average for 1000 grains weight, which reached 23.04 (g), followed by the FR21 and FR17 whose averages reached 21.43 g and 21.17 (g) respectively, under the level of salinity 7.5 dS.m⁻¹, while the genotype FR17 gave an average of 19.93 (g), without significant difference from genotype FR21 which gave an average of 19.26 (g) under salt level 15 dS.m⁻¹, superior to the rest genotype, while the lowest average was recorded for the trait with the Jasmine variety under salinity levels of S1, S2 and S3, which reached 15.43, 14.07 and 11.99 (g), respectively. These results are in agreement with [12].

### Table 6. Effect of genotypes and salinity levels and their interaction on productive tillers.

| Genotype | S1  | S2  | S3  | Average of genotype |
|----------|-----|-----|-----|---------------------|
| FR1      | 10.50 | 8.55 | 7.94 | 9.00 |
| FR2      | 14.28 | 10.77 | 6.89 | 10.65 |
| FR12     | 15.06 | 13.67 | 11.61 | 13.45 |
| FR15     | 11.22 | 9.67 | 4.94 | 8.61 |
| FR16     | 11.33 | 9.45 | 6.55 | 9.11 |
| FR17     | 8.94 | 7.33 | 6.86 | 7.71 |
| FR18     | 11.44 | 7.78 | 6.50 | 8.57 |
| FR21     | 9.03 | 8.11 | 7.11 | 8.08 |
| FR25     | 10.00 | 8.33 | 7.22 | 8.52 |
| FR27     | 11.22 | 9.56 | 8.28 | 9.69 |
| Jasmine  | 14.22 | 9.72 | 7.33 | 10.42 |
| Anber -33 | 12.22 | 9.78 | 8.58 | 10.19 |
| Pokkali  | 9.11 | 8.11 | 7.00 | 8.07 |
| Average of salinity levels | 11.43 | 9.29 | 7.45 |

### Table 7. Effect of genotypes and salinity levels and their interaction on 1000 grains weight (g).

| Genotype | S1  | S2  | S3  | Average of genotype |
|----------|-----|-----|-----|---------------------|
| FR1      | 19.71 | 16.85 | 13.77 | 16.78 |
| FR2      | 19.89 | 17.77 | 16.17 | 17.94 |
| FR12     | 18.70 | 18.20 | 16.72 | 17.87 |
| FR15     | 20.93 | 19.24 | 17.11 | 19.09 |
| FR16     | 20.23 | 18.53 | 15.21 | 17.99 |
| FR17     | 22.73 | 21.17 | 19.93 | 21.28 |
| FR18     | 17.84 | 16.53 | 13.77 | 16.05 |
| FR21     | 23.04 | 21.43 | 19.26 | 21.24 |
| FR25     | 18.20 | 16.78 | 14.06 | 16.35 |
| FR27     | 19.63 | 18.93 | 16.07 | 18.21 |
| Jasmine  | 15.43 | 14.07 | 11.99 | 13.83 |
| Anber -33 | 20.77 | 17.60 | 13.32 | 17.23 |
| Pokkali  | 21.31 | 17.93 | 16.93 | 18.72 |
| Average of salinity levels | 19.88 | 18.08 | 15.72 |

### Table 6. Effect of genotypes and salinity levels and their interaction on productive tillers.

### Table 7. Effect of genotypes and salinity levels and their interaction on 1000 grains weight (g).

#### 3.6 Grain yield per plant (g)

The results in (table 8) indicate a significant difference between the genotypes in the grain yield per plant, the FR12 genotype was superior to the other genotypes, which gave the highest average of grain yield which reached 22.51 (g), that is due to its superiority in the two components of the yield the number of productive tillers (Table 6) and the number of grains filled per panicle (Table 7), also, the genotype FR17 ranked second in grain yield, which amounted to 17.73 (g), without significant difference from genotype FR21, which averaged 16.76 (g), due to its superiority in 1000 grains weight (Table 7) although the composition The FR21 genotype gave a value close to the 1000 grains weight which made the difference between them not significant, while, the Jasmine variety gave the lowest yield per plant, which reached 6.50 (g), without significant difference from the genotype FR18 and FR25, which gave an average of 7.04 and 7.23 (g), respectively.
The results of the same table showed a significant decrease in the grain yield per plant with the increase of salinity levels, starting from 7.5 dS/m, the first level of salinity S1 gave the highest grain yield per plant amounting to 18.48 (g), while the percentage reduction in yield at levels S2 and S3 were 37.82 and 62.77%, respectively.

As for interaction between the factors, the FR12 genotype gave the lowest percentage reduction of grain yield per plant at the level of salinity 7.5 and 15 dS/m, amounted to 19.05% and 42.70% respectively, followed by genotype FR21, which gave a reduction per cent at the level of salinity 7.5 and 15 dS/m, which amounted to 24.22% and 45.53% respectively, while the high percentage reduction in grain yield for genotype FR25 at salt level of 7.5 and 15 dS/m was 57.01% and 82.23 % respectively, the reason for the clear superiority of salinity levels and their interaction is due originally to their superiority in the number productive tillers per plant (Table 6), the number of grains per panicle (Table 7) and 1000 grains weight (Table 8), these results agreement with finding by [14].

| Genotype | Level of salinity | Average of genotype |
|----------|------------------|---------------------|
| FR1      | S1   | 14.84 | 6.65 | 10.39 |
|          | S2   | 9.67  | 4.17 | 12.28 |
| FR2      | S1   | 20.90 | 16.24| 22.51 |
|          | S2   | 11.76 | 4.74 | 11.72 |
| FR12     | S1   | 28.34 | 11.33| 22.51 |
|          | S2   | 22.94 | 7.67 | 8.60  |
| FR15     | S1   | 17.64 | 11.33| 17.73 |
|          | S2   | 12.78 | 4.37 | 8.00  |
| FR16     | S1   | 13.77 | 7.67 | 8.60  |
|          | S2   | 7.67  | 3.90 | 7.04  |
| FR17     | S1   | 24.92 | 11.33| 17.73 |
|          | S2   | 16.94 | 4.37 | 8.60  |
| FR18     | S1   | 12.05 | 3.90 | 7.04  |
|          | S2   | 5.17  | 3.90 | 7.04  |
| FR21     | S1   | 21.72 | 11.83| 16.67 |
|          | S2   | 16.46 | 3.42 | 7.23  |
| FR25     | S1   | 17.64 | 3.05 | 9.84  |
|          | S2   | 9.32  | 3.33 | 6.50  |
| FR27     | S1   | 10.78 | 3.33 | 6.50  |
|          | S2   | 5.39  | 3.33 | 6.50  |
| Jamine   | S1   | 24.80 | 7.28 | 14.95 |
|          | S2   | 12.78 | 3.33 | 6.50  |
| Anber -33| S1   | 20.49 | 9.17 | 14.24 |
|          | S2   | 13.06 | 3.33 | 6.50  |
| Pokkali  | S1   | 18.48 | 6.88 | 13.08 |
|          | S2   | 11.49 | 3.33 | 6.50  |
| Average  | S1   | 18.48 | 6.88 | 13.08 |
|          | S2   | 11.49 | 3.33 | 6.50  |

3.7 Physiological traits in identified tolerant genotypes

The results indicated in (Table 9) that the percentage of sodium in the shoots of the tolerant genotypes FR12 and FR21 were lower than the control varieties (Amber-33 and Jasmine - salinity sensitive), and the salinity-tolerant control variety is Pokkali, Which indicates that the high content of sodium shows great damage in the leaves, which affects the metabolic processes in the plant under conditions of salt stress, The tolerant genotypes FR12 and FR21 recorded the lowest sodium ion content in the shoot, which was 0.38 and 0.42% compared to the control varieties Jasmine, Amber-33 and Pokkali, which gave averages of 1.01, 0.90 and 0.66%, respectively.

While the potassium content increased in the tolerant genotypes FR12 and FR21, which gave averages of 1.23 and 1.22% compared to the above control varieties, which gave 1.00, 0.85 and 1.20 %, respectively. With regard to the ratio of K⁺/Na⁺, the genotypes FR12 and FR21 registered the highest ratio of K⁺/Na⁺, which amounted to 3.30 and 2.95% compared to the control varieties of Jasmine, Anber-33 and Pokkali, which gave rates of 1.37, 1.15 and 1.85% respectively, which are necessary for the nature of the process of plant metabolism and cell cytoplasm. It should be noted that the genotypes tolerant to salt stress were followed salt exclusion mechanism of salinity tolerance. With respect to relationship between Na⁺ and K⁺, it is reported that the K⁺ uptake by plant is severely affected by presence of Na⁺ due to its similar physiochemical properties, the Na⁺ competes with K⁺ in plant uptake. The actual mechanism involved in that is the Na⁺ causes membrane depolarization makes it difficult for K⁺ uptake by K⁺ inward rectifying channels and increases the K⁺ leakage from the cell by activating K⁺ outward rectifying channels [15].
Characterizing and diagnosing genotypes at the level of DNA using polymerase chain reaction techniques averages revealing their fingerprint, It is the way in which the amplified of DNA fragments of the studied genotypes or varieties are distribute. It is the way in which the amplified of DNA fragments of the studied genotypes or varieties are distribute 

3.8 Investigation of the OsHKT4 and OsHKT6 genes responsible to some salt tolerance mechanisms in rice

| Genotype | Mean of genotype | Level of salinity |
|----------|------------------|-------------------|
| FR1      | 20.7             | 0.91              |
| FR2      | 20.7             | 0.91              |
| FR12     | 20.7             | 0.91              |
| FR15     | 20.7             | 0.91              |
| FR16     | 20.7             | 0.91              |
| FR17     | 20.7             | 0.91              |
| FR21     | 20.7             | 0.91              |
| FR25     | 20.7             | 0.91              |
| FR27     | 20.7             | 0.91              |
| Jasmine  | 20.7             | 0.91              |

Table 9. Estimates of physiological traits related to salinity tolerance in identified tolerant landraces under saline condition

| Genotype | Level of salinity |
|----------|-------------------|
| L.S.D (0.05) | 0.07 | 0.06 | 0.11 |
| Genotype | Level of salinity |
| L.S.D (0.05) | 0.07 | 0.06 | 0.11 |

| Genotype | Level of salinity |
|----------|-------------------|
| L.S.D (0.05) | 0.07 | 0.06 | 0.11 |
| Genotype | Level of salinity |
| L.S.D (0.05) | 0.07 | 0.06 | 0.11 |
**Conclusion**

The study revealed that different levels of salinity had significant effects on the plant height, flag leaf area, number of productive tillers, panicle length, 1000-grain weight, yield per plant and physiological traits K⁺ content, Na⁺ content and K⁺/Na⁺. The tolerant genotype FR12 and FR21 to salinity level of 7.5 dS/m. There were a 19.05 and 24.21% yield reduction of rice under saline condition compared to non-saline condition. As for molecular analysis above genotype show banding on gel electrophoresis that mean contain to OsHKT4 and OsHKT6.

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