

# Contents

<b>1.</b>	<b>Introduction</b>	<b>1</b>
<b>2.</b>	<b>Review of literature</b>	<b>4</b>
2.1.	Utilization of anther culture technique in rice breeding Program	4
2.2.	Salinity and salinity tolerance in rice	7
2.3.	Rice performance under salinity conditions	9
2.3.1.	Agronomic characters	9
2.3.2.	Yield and its components	9
2.4.	Genetic variability	10
2.5.	Phenotypic correlations	12
2.6.	Path co-efficient analysis	15
2.7.	Marker assisted selection in breeding rice for salinity tolerance	15
2.7.1.	Total Soluble Proteins (TSP)	15
2.7.2.	Microsatellite markers (SSR)	18
2.7.3.	Intron-exon Splice Junctions (ISJ)	20
<b>3.</b>	<b>Material and methods</b>	<b>24</b>
3.1.	Plant materials	24
3.2.	Anther culture technique	24
3.3.	Evaluation of the tested genotypes	28
3.3.1.	Field evaluation (normal conditions)	29
3.3.2.	Lysimeter evaluation (salinity controlled conditions)	29
3.4.	Studied characters	30
3.4.1.	Agronomic characters	30
3.4.2.	Yield and its components characters	31
3.4.3.	Salinity index (SI%)	32
3.5.	Statistical analysis	32
3.5.1.	Analysis of variance	32
3.5.2.	Estimation of component variances	33
3.5.3.	Estimation of phenotypic correlations	35
3.5.4.	Path co-efficient analysis	35
3.6.	Total soluble protein preparation	36
3.6.1.	Sample extraction	37
3.6.2.	Polyacrylamide gel preparation (PAGE)	37
3.6.3.	Staining and analysis	38
3.7.	Microsatellite markers (SSR) and Intron-exon splice junctions (ISJ) protocols	38
3.7.1.	DNA isolation and quantification	38
3.7.2.	SSR protocol	40
3.7.3.	ISJ protocol	41
3.7.4.	Electrophoresis, staining and analysis	41
3.7.5.	Phylogenetic tree construction	42

<b>4.</b>	<b>Results and discussions</b>	<b>43</b>
4.1.	Evaluation of the genotypes	43
4.1.1.	Field evaluation under normal conditions	43
4.1.1.1.	Mean performance	43
4.1.1.1.1.	Agronomic characters	43
4.1.1.1.2.	Yield and its component characters	48
4.1.1.2.	Genetic variability under normal conditions	53
4.1.1.3.	Phenotypic correlation coefficients for normal conditions	56
4.1.1.4.	Path co-efficient analysis under normal conditions	59
4.1.2.	Lysimiter evaluation	64
4.1.2.1.	Mean performance	64
4.1.2.1.1.	Agronomic characters	64
4.1.2.1.2.	Yield and its components characters	78
4.1.2.2.	Genetic variability under Lysimiter conditions	88
4.1.2.3.	Phenotypic correlation coefficients	92
4.1.2.4.	Path co-efficient analysis	96
4.1.3.	Salinity index	101
4.2.	Biochemical and molecular Analysis of Genetic Diversity of the Tested Genotypes	107
4.2.1.	Polymorphism of total soluble protein (TSP)	107
4.2.2.	Polymorphism of microsatellite markers	111
4.2.2.1.	RM 223	111
4.2.2.2.	RM 315	113
4.2.2.3.	RM 208	113
4.2.2.4.	RM 224	113
4.2.2.5.	RM 276	119
4.2.3.	Polymorphism of ISJ markers	119
4.2.3.1.	ISJ-5	119
4.2.3.3.	ISJ-7	123
4.2.3.2.	ISJ-9	126
4.2.4.	Genetic similarity and phylogenetic tree	129
<b>5.</b>	<b>Summary</b>	<b>134</b>
<b>6.</b>	<b>References</b>	<b>144</b>
<b>7.</b>	<b>Arabic summary</b>	

## Abbreviations

<b>DAH</b>	Days to heading
<b>DHLs</b>	Doubled haploid lines
<b>FLA</b>	Flag leaf area
<b>GA%</b>	Genetic advance as percentage of mean
<b>GCV</b>	Genotypic coefficient of variation
<b>GS</b>	Genetic advance
<b>GYP</b>	Grain yield plant <sup>-1</sup>
<b><i>h</i><sup>2</sup></b>	Heritability in broad sense
<b>HAI</b>	Harvest index
<b>ISJ</b>	Intron-exon Splice Junctions
<b>NPT</b>	Number of productive tillers plant <sup>-1</sup>
<b>PAL</b>	Panicle length
<b>PAW</b>	Panicle weight
<b>PCV</b>	Phenotypic coefficient of variation
<b>PFP</b>	Pollen fertility percentage
<b>PLH</b>	Plant height
<b>R%</b>	Reduction percentage
<b>RCBD</b>	Randomized Complete Block Design
<b>RRTC</b>	Rice Research & Training Centre
<b>SI%</b>	Salinity index
<b>SSP</b>	Spikelet sterility percentage
<b>SSR</b>	Simple sequence repeats
<b>SWP</b>	Straw weight plant <sup>-1</sup>
<b>T1</b>	Treatment 1 (tap water)
<b>T2</b>	Treatment 2 (4000 ppm)
<b>T3</b>	Treatment 3 (6000 ppm)
<b>TCC</b>	Total chlorophyll content
<b>TGW</b>	Thousand grain weight
<b>TSP</b>	Total soluble protein
<b><math>\sigma^2_g</math></b>	Genetic variation
<b><math>\sigma^2_{ph}</math></b>	Phenotypic variation

## Summary

The present investigation was carried out at the experimental farm and Rice Biotechnology Lab of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt during 2003-2005 seasons.

**The objectives of the present investigation were to:**

- 1) Develop doubled haploid lines of rice tolerant to salinity using anther culture technique.
- 2) Evaluate the produced lines for agronomic and yield and its component characters under normal soil and artificial saline soil conditions (Lysimiter).
- 3) Estimate genetic variability, phenotypic correlation coefficients and path co-efficient under normal and Lysimiter conditions.
- 4) Estimate salinity tolerance index (**SI %**) of the studied genotypes for all studied characters under Lysimiter conditions.
- 5) Study the genetic diversity among twenty two selected genotypes by using total soluble protein (TSP), simple sequence repeats (SSR) and intron-exon splice junctions (ISJ) techniques.

**To achieve these objectives**, four Egyptian rice varieties *i.e.* Giza 177, Sakha 101 (two salt susceptible varieties), Sakha 104 (moderately salt tolerance variety) and Giza 159 (tolerant variety) were utilized. The parental varieties were crossed using half-diallel design and six crosses were obtained. The F<sub>1</sub>-seeds were grown and anthers of F<sub>1</sub> plants were used to develop the doubled haploid lines (DHLs) using anther culture technique. The **FJ** medium was used for callus induction while **MS** medium was used for plant regeneration. One hundred doubled haploid lines (DHLs) were optioned.

The one hundred lines, the four parents and two salinity check varieties were grown and evaluated under normal conditions and Lysimeter conditions (tap water, 4000 ppm and 6000 ppm). Thirteen agronomic and yield and its components characters were measured for all genotypes.

The analysis of variance was computed for the studied characters. The genetic components *i.e.* phenotypic variation ( $\sigma^2_{ph}$ ), genotypic variation ( $\sigma^2_g$ ), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense ( $h^2$ ) and genetic advance (GA%) were computed. Also, phenotypic correlation coefficients between all pairs of the studied characters were computed and path coefficient analysis was performed for all characters.

Twenty two genotypes were selected depending on their yield under different treatments and the genetic diversity among them was studied by using three types of markers *i.e.* total soluble protein banding patterns (TSP), five SSR markers and three ISJ markers.

### **The obtained results could be summarized in the following points**

#### **1. Field evaluation (Normal conditions)**

- 1) The mean square estimates revealed highly significant variations among genotypes for all studied characters.
- 2) For agronomic characters, wide range of variations was exhibited among the studied genotypes. The mean values were ranged from 40.6 to 53 mg ds<sup>-2</sup> for total chlorophyll content, from 22.6 to 66.7 cm<sup>2</sup> for flag leaf area, from 83.7 to 97.7% for pollen fertility, from 84 to 121.7 for days to heading, from 78.5 to 130.4 cm for plant height, from 19 to 26.7 cm for panicle length and from 35.6 to 69 gm for straw weight plant<sup>-1</sup>. the best mean values were scored by DHL 5 and Sakha 101 for chlorophyll content, TCCP266 and DHL 63 for flag leaf area, DHL 94 and TCCP266

for pollen fertility percentage, DHLs 43 and 36 for days to heading, DHLs 81 and 84 for plant height, TCCP266 and DHL 53 for panicle length and DHLs 59, 56 and 67 for straw weight.

- 3) For yield and its components characters, wide range of variations was observed among the studied genotypes. The mean values were ranged from 16.1 to 25.3 for no. of productive tillers plant<sup>-1</sup>, from 2.8 to 5.7 gm for panicle weight, from 22.2 to 32.8 gm for one thousand grain weight, from 1.9 to 15.5% for sterility percentage, from 22.6 to 51.2 gm for grain yield plant<sup>-1</sup> and from 30.5 to 48.9% for harvest index. The best mean values were scored by IR 29, DHL 3 and DHL 80 for productive tillers plant<sup>-1</sup>, DHL 53 and DHL 40 for panicle weight, DHL 36 and TCCP266 for one thousand grain weight, DHL 4 and DHL 60 for sterility percentage, Sakha 101 and DHL 86 for harvest index and Sakha 101 and DHL 10 for grain yield plant<sup>-1</sup>.
- 4) For genetic parameters, it was observed that phenotypic coefficients of variability (PCV) were as expected higher than respective genotypic coefficients (GCV) for all traits. There were small differences between the two estimates suggesting that the environmental effects on the studied traits were not very large and this may facilitate selection based on them. The high estimates of heritability in broad sense were scored for days to heading, flag leaf area, plant height and pollen fertility. The moderate to high value of GCV,  $h^2$  and GA% were estimated for sterility percentage, flag leaf area and panicle weight suggesting that direct phenotypic selection based on these traits would be effective for varietal improvement under normal soil conditions.
- 5) For phenotypic correlation coefficients, grain yield plant<sup>-1</sup> was highly significant positive correlated with straw weight, productive tillers, panicle weight, one thousand grain weight and harvest index. Flag leaf

area was highly significant positive correlated with plant height, panicle length and panicle weight. Days to heading was positively and highly significant correlated with plant height, panicle length, straw weight and panicle weight, while it was negatively correlated with harvest index. Plant height was highly positive correlated with panicle length, straw weight, panicle weight, whereas it was significantly negative correlated with harvest index. Highly significant positive correlations were observed for panicle length with straw weight and panicle weight. No. of productive tillers had positive and high significant correlations with panicle weight and harvest index. One thousand grain weight had positive correlations with panicle weight and harvest index. Sterility percentage was significantly negative correlated with one thousand grain weight and harvest index.

- 6) For path coefficient analysis, the highest positive direct effects on grain yield were observed for straw weight plant<sup>-1</sup> and number of productive tillers, so that they seem to be very important traits affecting grain yield in rice. Also, panicle weight, one thousand grain weight, harvest index, had positive direct effects on grain yield. So, selection for these traits can be effective in improving rice grain yield. On the other hand, traits like sterility percentage and flag leaf area had negative direct effects on grain yield. Highly positive indirect effects were observed for days to heading, productive tillers, panicle weight and harvest index through straw weight. Meanwhile, straw weight plant<sup>-1</sup> exhibited highly negative indirect effect on grain yield through days to heading.

## **2. Lysimiter evaluation**

- 1) For mean squares, analysis of variance revealed highly significant differences among all genotypes for the studied traits under all treatments.

- 2) The all studied characters for all genotypes exhibited decreased estimations under salinity treatments (4000 ppm and 6000 ppm) compared with the control treatment (tap water) except for days to heading and sterility percentage.
- 3) The range between minimum and maximum values increased for most traits with increasing salt concentrations. Wide range of variations was observed for pollen fertility (20.7-84.3%), sterility percentage (14.3-61.8 %), plant height (50.6-97.4 cm), flag leaf area (7.3-47.3 cm<sup>2</sup>), days to heading (80-105 days) and harvest index (16.6-42.8%).
- 4) The lowest reduction percentages were revealed for chlorophyll content (10.6%), harvest index (17.1%) and plant height (19 %). Meanwhile, the highest reduction percentages were scored for panicle weight (61.4%), no. of productive tillers plant<sup>-1</sup> (57.2%) and grain yield plant<sup>-1</sup> (56.5%). On the other hand, the trait days to 50% flowering was increased with 1.9%, while sterility percentage was increased with 323.5%.
- 5) For agronomic characters, the highest mean performance estimates were scored by DHL 100 (48.3 mg ds<sup>-2</sup>) for chlorophyll content and TCCP266 for flag leaf area (47.3 cm<sup>2</sup>) with the lowest reduction percentage of 16.8%. For pollen fertility, TCCP266, DHLs 11, 17 and 58 scored the highest mean values. The earliest period for 50% flowering was revealed by DHL 85 while the longest period was revealed by DHL 4. For plant height, the highest mean value was observed for DHL 65 under 4000 ppm and 6000 ppm, while DHL 81 showed the lowest mean values under all treatments. The longest panicle length was scored by TCCP266 under all treatments and by DHL 16 among the doubled haploid lines. For straw weight, the highest values were recorded by TCCP266 under 4000 and 6000 ppm and by DHL56 among lines under 6000 ppm.



- 6) For yield and its components characters, the best mean performance estimates under 6000 ppm were revealed by DHL 72 for productive tillers, TCCP266 for panicle weight under all treatments, DHL 99 for one thousand grain weight under 4000 and 6000 ppm. The line DHL 8 scored the lowest mean value for sterility percentage and the highest mean value for harvest index. For grain yield plant<sup>-1</sup>, the check variety TCCP266 scored the highest mean value among parents, while DHL 16 exhibited the highest mean value among lines under 4000 and 6000 ppm.
- 7) For genetic variability, the phenotypic and genotypic variation increased for most traits compared with respective tap water treatment, the highest estimates were observed for plant height under tap water and pollen fertility under 4000 and 6000 ppm. The estimates of PCV were as expected higher than GCV and increased under 4000 and 6000 ppm for all traits except sterility percentage and days to heading. The highest estimates of GCV and PCV under 6000 ppm were recorded for grain yield plant<sup>-1</sup>. High estimates of  $h^2$  ranged between 80.26 and 93.68% were observed for flag leaf area, pollen fertility, plant height and sterility percentage under all treatments. Genetic advance as percentage of mean (GA%) increased under salinity treatments for most traits as like grain yield, panicle weight, straw weight, pollen fertility and flag leaf area. The high estimates of GCV,  $h^2$  and GA% were observed for flag leaf area, pollen fertility, sterility percentage, grain yield plant<sup>-1</sup> and straw weight suggesting that these traits could be improved by direct selection under saline soil conditions.
- 8) For phenotypic correlation coefficient, under all treatments, significant and high significant positive correlations were observed for (grain yield plant<sup>-1</sup> with flag leaf area, plant height, straw weight, panicle length,

panicle weight and harvest index), (flag leaf area with days to heading, plant height, panicle length, straw weight and panicle weight), (days to heading with straw weight), (plant height with panicle length, straw weight and panicle weight), (panicle length with straw weight and panicle weight) and (panicle weight with straw weight and one thousand grain weight). Under 4000 and 6000 ppm significant and high significant positive correlations were observed for (grain yield plant<sup>-1</sup> with pollen fertility, productive tillers, and one thousand grain weight), (pollen fertility with days to heading, panicle length, panicle weight and harvest index), (plant height with productive tillers and harvest index), (panicle length with productive tillers and harvest index), (straw weight with productive tillers and harvest index), (no. of productive tillers plant<sup>-1</sup> with panicle weight and harvest index), (panicle weight was highly significant positive correlated with harvest index), (one thousand grain weight with harvest index). On the other hand, under 4000 and 6000 ppm, significant and high significant negative correlations were observed for (sterility percentage and each of pollen fertility, plant height, panicle length, straw weight, productive tillers, panicle weight, one thousand grain weight and harvest index ) and (days to heading with one thousand grain weight).

- 9) For path co-efficient analysis, under all treatments, the traits straw weight, one thousand grain weight and pollen fertility had positive direct effects on grain yield, while negative direct effects were observed for sterility percentage and chlorophyll content. Under 4000 and 6000 ppm, panicle length and productive tillers had positive direct effects, whereas, days to heading and plant height had negative direct effects on grain yield. Panicle weight and harvest index had positive direct effects on grain yield under 6000 ppm. So, selection for straw weight, one

thousand grain weight, pollen fertility, panicle length, productive tillers, panicle weight and harvest index may be efficient in improvement for grain yield under saline soil conditions.

3. For salinity tolerance index values (**SI%**), among agronomic characters, the affected genotypes by salinity were flag leaf area, straw weight and pollen fertility with salinity index averages of 59.9, 56 and 55.1 %, respectively. among yield and its component characters, the most affected traits by salinity were panicle weight, grain yield plant<sup>-1</sup> and productive tillers with salinity index averages of 43.7, 43.3 and 38.8 %, respectively. For grain yield plant<sup>-1</sup>, salinity index values ranged between 17.4 and 78.6 %, the highest values were observed for TCCP266 (78.6%), DHL 69 (74.4%), DHL 7 (74.1), DHL 55 (73.1), Giza 159 (72.6%) and DHL 10 (69.7%).
  - 4.1. For total soluble protein (TSP), a total of twenty two bands with molecular weights ranged from 110 to 21 kda were identified in the twenty two selected genotypes. Four bands were monomorphic because presenting in all genotypes with the same intensities. The estimation of polymorphism percentage among the studied genotypes was 45.5%. The two bands TSP-7 and TSP-9 were found in all genotypes with the same intensity but they were absent in the two indica check varieties TCCP266 and IR 29. The presence of band **TSP-8**, with molecular weight of 50 kda, in TCCP266 with high intensity compared with the other genotypes might be linked to its tolerant to salinity.
  - 4.2. For microsatellite markers, five SSR markers were used i.e. RM 208, RM 223, RM 224, RM 276 and RM 315, all of them generated polymorphic bands among the genotypes except RM 315. A total of eleven alleles were detected among the twenty two genotypes. The number of alleles per locus ranged from one to three, with an average

of 2.2 alleles per locus. No linkage between the SSR markers used and the salt tolerance in the genotypes under study. The level of polymorphism generated was not enough to study the diversity between the selected genotypes, so that, another DNA marker was used to find more polymorphism between them.

- 4.3. For ISJ marker, Three **ISJ** Primers *i.e.* **ISJ-5**, **ISJ-7** and **ISJ-9** were used to evaluate the genetic diversity among the selected genotypes. The number of DNA fragments which generated by using these three primers were ranged between eight fragments for **ISJ-7** and seventeen fragments for **ISJ-5**. All of the three **ISJ** primers generated polymorphic bands. A total of thirty nine DNA fragments were generated. Seven fragments were monomorphic *i.e.* **ISJ-5.9**, **ISJ-5.13**, **ISJ-5.16**, **ISJ-7.7**, **ISJ-9.6**, **ISJ-9.7** and **ISJ-9.10** while the remaining thirty two fragments were polymorphic. The polymorphic ratio ranged between of **34.22%** to **47.73%** with an average of **38.81%**. Four DNA fragments *i.e.* **ISJ-5.2**, **ISJ-7.4**, **ISJ-7.5**, **ISJ-9.9** were found only in the two check varieties TCCP266 and IR 29 which are indica type, so these four fragments might be linked to the indica type. On the other hand, the DNA fragment **ISJ-5.17** was found only in all genotypes with japonica type, so it might be linked to japonica type. The DNA fragment **ISJ-9.1** was only present in Sakha104, while, the fragment **ISJ-9.2** was only present in the salt tolerant check variety TCCP266.
5. For genetic similarity, The lowest similarity percentage (48%) was present between the TCCP266 and DHL 67, while, the highest similarity percentage (100%) was observed for DHL 34 and DHL 37 which are derived from the same cross Giza 177 x Sakha 104. the cluster analysis grouped all genotypes into two major branches in the dendrogram with

---

59% similarity. One branch represents the subspecies, indica rice, and the other branch represents the subspecies, japonica rice. Below the main indica branch in the dendrogram, the two indica varieties TCCP266 and IR 29 grouped into one cluster, A at about 76% similarity. Below the main japonica branch in the dendrogram, most genotypes grouped into seven clusters, B1, B2, B3, B4, B5, B6 and B7, at about 89, 84, 80, 94, 88, 92 and 85% similarity, respectively.