CELL MEMBRANE STABILITY, PHOTOSYNTHETIC RATE AND ASSOCIATED TRAITS AS AN INDICATOR OF SALINITY TOLERANCE IN SOME RICE CULTIVARS (*Oryza sativa, L.*)

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Abstract

Stability of cell membrane has long been recognized as an important factor in determining a grown plant species adaptability to saline conditions.Therefore, three rice cultivars,i.e. (Giza 177 as sensitive one , Giza 178 tolerant one and M 201 an American sensitive one) and three salt levels of. (0.98, 4.0 & 8.0 ds/m) with six replication were used in this study . The present study was carried out at US Salinity Lab, Riverside, California, USA during year 2000. Cell membrane, photosynthetic rate, stomatal conductivity, chlorophyll content, leaf area, leaf vapor pressure deficit and transpiration rate were determined at 40 DAS.

The major results could be summarized as following, salt level had high significant effect on electrolyte leakage (EL%), where, EL was gradually increased with increasing salt level by submergence leaf discs in saline water or only deionized distilled water. The three rice cultivars had different performance with regards to EL. Giza 177 gave the highest value of EL while Giza 178 produced the lowest value of EL followed by M 201 which means the sensitive cultivars (Giza 177 and M-201) seems to have more cell injury. The interaction effect between cultivars and salt levels was significant concerning EL in both trials. NaCl salinity at various levels had unfavorable effect on photosynthetic rate, transpiration rate, stomatal conductivity, leaf area and chlorophyll content. Also, air to leaf water vapor deficit pressure increased as salt levels increased up to 8.0 dS/m^2. Varietal differences in the above mentioned traits were recorded. Also, the interaction between rice cultivars and salt levels had a significant effect on photosynthetic rate, leaf area and chlorophyll content.

INTRODUTION

Sustainability of rice to salinity stress including plant survival and production is conditioned by very complex mechanisms where, it differs from one developmental stage to another, varieties to another and year to year. Many studies revealed that cell membrane as initial site of stress injury. It is very important to mention that the function and structure of plant cell membrane are drastically damaged by various environmental stresses (Mckerise and Tomes, 1980 and Mckerise *et al.* 1982). Changes in the electrolyte leakage and electrical impedance have been determined to investigate cell membrane stability. Moreover, the electrolyte leakage will differ coinciding with the membranes' abilities to take up and retain solutes, and therefore will reflect stress-induced changes in both membrane potentials and membrane permeability. Rice is sensitive crop for salinity and therefore , it is very important to improve rice cultivars to cope with the hazards of salt stress. Detecting which physiological traits are most connected with the mechanism of salinity tolerance and transfer it from tolerant cultivar to sensitive one is aimed in this study. A few studies were carried out to measure membrane integrity in rice by drought and heat tolerance both of which was found to be efficient in estimating stress tolerance of several crops plant involving wheat (*Triticum aestivum*, L.) Premachandra and Shimada (1987) and Blum and Edercon (1981), barley (*Hordeum valgure*, L.) (Blum and Ebercon, 1981) Orchardgrass (*Dactylis glomerata*, L.) (Premachanda and Shimada 1987b), Maize (*Zea mays*, L.) (Premachandra *et al.,* 1989) and Rice (*Oryza sativa*, L.) Bewley (1979). The later researcher reported the critical role of cell membrane stability under moisture stress as a major component of drought tolerance. The injury rate of cell membrane by salinity may be estimated by electrolyte leakage from the cells. Blum and Ebercon (1981) pointed out significant varietal differences in wheat with regards to electrolyte leakage under drought stress. Agarie *et al* (1995) found that in desiccation test with polyethylene glycol (PEG), electrolyte leakage (EL) increased from 10% to 80% with successive increasing from 20% to 60% and also with increasing the time of exposure. At the same time EL of the leaf discs was increased with severe water deficit ,indicating that the physiological status of the plant itself can be affected by this technique. Asch *et al.* (1997), Sultana *et al.* (1999) emphasized that salt stress significantly restricted the photosynthetic rate, transpiration rate, stomatal conductivity, leaf area and relative chlorophyll content but enhance leaf vapor pressure deficit. Also, their results demonstrate varietal differences in aforementioned traits. Asch *et al* (2000) recorded varietal differences in photosynthetic rate and its associated traits due to different varietal salt tolerance .

In the present study, the main aim was to explore the possibilities of cell membrane stability measurement as indicator of salinity tolerance of rice (*Oryza sativa*, L.) and investigate the physiological behavior of different rice cultivars under salt stress in the green house .

MATERIALS AND METHODS

Rice cultivars i.e. Giza 177(salt sensitive one), Giza 178 (salt tolerant one) and M 201 (American sensitive one) were grown in the sandy tanks with circulating irrigation system using Yoshid's standard solution (Yoshida *et al.* 1976). Three salt levels i.e. 0.98, 4.0 and 8.0 dS/m were applied as NaCl+CaCl. The culture solution and E.C were adjusted weekly as well as pH of the solution was adjusted to 5.0 to 5.5 with H_2SO_4 over growth duration. The leaf sample were taken from the fourth fully expanded leaves .Split plot design was followed with six replication where the three rice cultivars were randomly distributed in the main plots and the three salt levels were arranged in the sub plots. The data analysis of split plot design was used according to (Gomez and Gomez, 1984).

A- Measurement of electrolyte leakage

1- Saline water test

The leaf sample were cut into one cm pieces. Thirty five leaf pieces were put into 100ml flask and carefully washed with deionized distilled water three time to remove surface adhered electrolytes. The washed leaf pieces were submerged in 30 ml of different saline solution which coincide with those used in the green house experiment (0.98, 4.0 and 8.0 dS/m). The leaf discs were kept at 10°C for 24 hours in the dark to minimize secondary effects. After 24 hours, the leaf pieces were taken out and quickly washed with deionized distilled water three times. Thirty ml of deionized distilled water were added and it were kept again at 10°C for 24 hours in the dark. After 24 hour the flasks were warmed to 25 °C then shaken well, and the electrical conductivity was measured, the leaf tissue was killed by autoclaving for 15 minutes to release all ions from the tissue, then cooled to 25 °C and immediately electrical conductivity was measured (Agarie *et al.,* 1995).

Electrolyte leakage was calculated using (*Eci/Ect*) X 100 according to (Ismail and Hall, 1999), where Eci is the initial electrical conductivity and the Ect is the final electrical conductivity after cell killing .

2- Deionized distilled water test

The same steps of the above test were followed replacing the saline solution with was replaced by the deionized distilled water and used with leaf discs from different salt level which were applied in the green house. After 24 hours the electrical conductivity was done.

It is very important to mention that the age of leaf and the date of sampling have great effect on electrolyte leakage in terms of cell membrane stability.

B- Photosynthetic rates and its parameter

At 40 days after sowing (DAS) leaf area/plant was measured by leaf area meter. Relative leaf chlorophyll content was measured on the same hills using a SPAD meter (Minolta) on the youngest fully-developed leaves. Leaf photosynthetic rate and its associated parameter were measured with the Photosynthesis System Licor 6400 at 40 DAS on the top two fully-expanded leaves that was on same hills in which chlorophyll contents were estimated.

RESULTS AND DISCUSSION

There is no doubt that salinity has detrimental effect on the cell membrane and its stability because of ion imbalance , particularly due to more Na ion uptake. The measurement of electro conductivity of aqueous media containing leaf discs were taken from plants stressed by various salt levels .Moreover the discs itself were treated with saline water at the same salt levels as in the greenhouse or treated only with deionized distilled water .

A- Results of submerging leaf discs in saline water plus salts levels in the green house

In this study, varietal differences were detected regarding electrolyte leakage where, significant differences were recorded among Giza 177 (Egyptian sensitive one), Giza 178 (Egyptian tolerant one) and M 201 (American cultivar). At the same time no significant differences were observed between Giza 178 and M 201. Giza 177 gave the highest value of electrolyte leakage while Giza 178 gave the lowest value of electrolyte leakage table (1). The inferiority of Giza 177 regarding electrolyte leakage might be due to much Na uptake as it was recorded from the unpublished data taken from the same experiment to determine Na and K uptake. Sodium (Na) probably damaged the cell membrane and thereby affected its stability. These results were in agreement with those reported by Blume and Ebercon (1981) on wheat subjected to drought. Concerning salt levels effect, it was reported that salt stress had highly significant effect on electrolyte leakage where El successively increased with increasing salt levels and also with increasing salt levels of submerging water up to 8.0 dS/m. These data were in harmony with those reported by Agarie *et al* (1995) with rice under drought using PEG .

The interaction between rice cultivars and salt levels exerted marked effect on the electrolyte leakage. The highest value of electrolyte leakage was produced by Giza 177 subjected to higher salt levels of 8.0, while the lowest value of electrolyte leakage was obtained when M 201 received no salt. It is very important to mention that within the control level, no significant differences were noticed among the three rice cultivars. In the second level of salt (4.0 dS/m), Giza178 and M201 came in the same level of significance while electrolyte leakage of Giza177 under moderate salt level of 4.0 dS/m came at the same level of significance with those were produced by Giza 178 under the higher salt level (8.0dS/m). Under the higher salt level (8.0dS/m), highly varietal differences were detected regarding electrolyte leakage. Giza177 came at the first rank indicating its inferiority and M 201came in the second rank. Giza 178 occupied the last rank fig. (1). Thereby Giza 178 had considerable ability to keep its cell membrane healthy under salt stress. The obtained data are in a good harmony with those reported by Blum and Ebercon (1981).The values of EL% were still low due to the salt levels which weren't so high to induce more damage in the cell membrane .The results obtained at 4 dS/m confirmed that ,the three varieties had the same behavior regarding their cell membrane .The previous findings completely agreed with Na^+ and K^+ contents (unshown data obtained in our lab.).

Table 1. Averages of electrolyte leakage EL %as affected by some rice cultivars and three salt levels with and without pretreated and pre -treated

Trait / Treatments	With pre-treated	EL % without pre-treated
Cultivars		
Giza 177	11.96A	9.97 A
Giza 178	8.56 C	7.07 B
M-201	10.77B	7.87 B
F test	**	**
Salt stress dS/m		
0.98	3.93 C	4.26 C
4.0	8.37 B	7.59 B
8.0	18.99 A	13.06 A
F test	**	**
Interactions	**	**

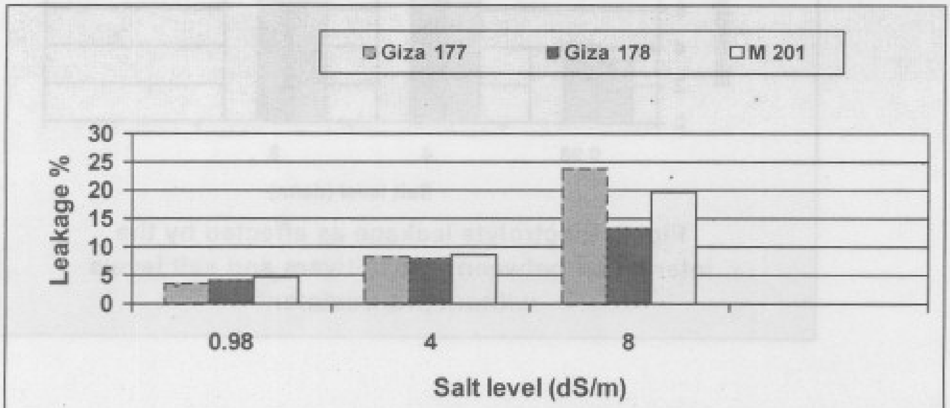

Fig. 1. Electrolyte leakage % as affected by the interaction between rice cultivars and salt levels with pre-treatment

B- Results of deionized distilled water submerging

To confirm the data which was obtained with saline water corresponding to the same salt levels used in the greenhouse. It was found that the three rice cultivars performed the same trend observed with saline water. Also, the salt levels had similar to results as those produced by the previous treatment of submerging of leaf discs in saline water(table1). The interaction between cultivars and salt levels had reasonable significant effect on percentage of electrolyte leakage where Giza 177 recorded the highest and Giza 178 gave the lowest value of electrolyte leakage when both were grown at 8.0dS/m. Furthermore, within the control level of 0.98 dS/m and salt level of 4.0dS/m, no significant differences were detected among the three rice cultivars regarding electrolyte leakage(fig,2). From the former data, it is clear that under the higher salt level the electrolyte leakage differences among cultivars were recognized . Submerging leaf discs in saline solution is proved to be unimportant tool investigating stability cell membrane by electrolyte leakage of rice. It could be concluded also that drastic damage of Na on the cell membrane stability could be provided valuable information about the behavior of rice plants against environmental stresses such as, salt stress. These finding are in a good agreement with those reported by Agarie *et al.* (1995).

The above mentioned findings clearly demonstrate that cell membrane stability under salinity stress is useful tool for rice breeding program .

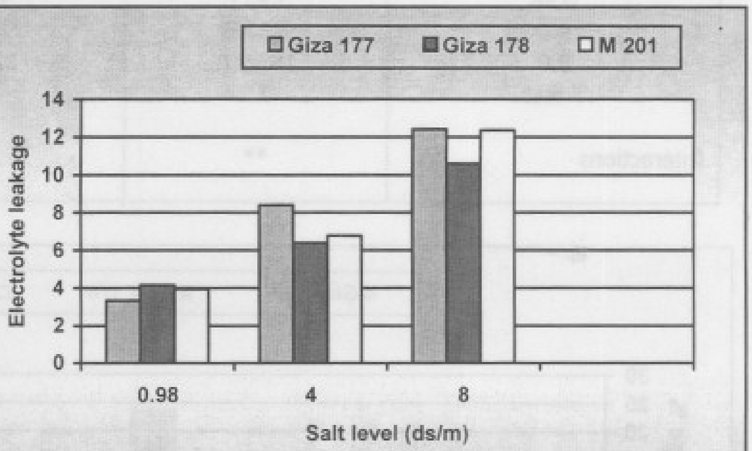

Fig. 2. Electrolyte leakage as affected by the interaction between rice cultivars and salt levels without pre-treatment

II- Photosynthetic rates and its associated traits

Referring to tables 2, 3 and 4, high varietal differences were detected in photosynthetic rate, LA, transpiration rate, stomatal conductivity, vapor pressure deficit, relative chlorophyll content. Giza 178 scored first rank in photosynthetic rate, transpiration rate, stomatal conductivity while it gave the lowest values of LA, vapor pressure deficit and relative chlorophyll content (SPAD). The highest values of LA and relative chlorophyll content were produced by M-201. Both cultivars Giza 177 and M-201 had the same values of vapor pressure deficit. Also the couple sensitive cultivars were on a par in most of studied characters, for instance, photosynthetic rate, stomatal conductivity and relative chlorophyll content. Giza 177 gave the least values of leaf area (LA), transpiration rate and stomatal conductivity while the least value of photosynthetic rate was given by M-201. In addition, M 201 gave the largest leaf. It seems that the photosynthetic rate and associated parameters of the three tested rice cultivars are in comparable to their cell membrane stability . Asch *et al* (1997) and Asch *et al* (2000) found varietal differences regarding the tested traits. It seems that the varieties with higher photosynthetic rate were found to have higher transpiration and stomatal conductivity and lower air to water vapor pressure deficit. The previous mentioned finding was true with the salt-tolerant cultivars of Giza 178. Also it was shown that chlorophyll content and leaf area had no effect on photosynthetic rate of studied cultivars.

Concerning the effect of salt stress on photosynthetic and its associated trait, it was found that increasing salt levels significantly reduced leaf area, photosynthetic rate, transpiration rate, stomatal conductivity and relative chlorophyll content and increased leaf water vapor pressure deficit up to the higher salt level of 8.0 dS/m. The higher salt level of 8.0 dS/m produced the lowest values of all studied traits except water vapor pressure deficit which was the highest value(tables 2.3&4). The highest values of photosynthetic rate, leaf area, transpiration rate, stomatal conductivity and the lowest value of water vapor deficit pressure were produced by the lower salt level of 0.98 ds/m. The current findings were in conformity with those reported by Sultana *et al* (1999). Also, it is evident that high salinity results a decline in photosynthetic parameters including osmotic and leaf water potential, transpiration rate, leaf temperature and relative water content thereby lower photosynthetic rate. In addition, higher water vapor pressure deficit reduced photosynthetic rate and that might be due to higher water vapor pressure deficit due to increased salt accumulation in plant tissue (Asch,et al 1999). In addition, Asch *et al.* (2000) reported that to get an observable reduction in photosynthetic rate and its associated parameters, sodium concentration has to be high enough. Also, they added that low sodium concentration may increase photosynthetic rate .It was proved that the leaf area and stomatal conductance were more severely affected by salt stress than other traits .Where, both of them reached to their Ic50 values at the salt level of 8.0 ds/m while the others didn't reach. On the controversy the air to leaf water vapor pressure deficit increased when the salt levels were increased indicating its hazardous effect on plant grown under salt stress

Table 2. Mean of leaf area (LA) cm^2/plant and photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) as influenced by various varieties and salt levels

Trait / Treatments	LA	Photosynthetic rate
Varieties :		
Giza 177	29.31 c	12.54 b
Giza 178	32.42 b	13.38 a
M 201	38.55 a	11.98 b
LSD 0.05	**	**
Salt levels dS/m :		
0.98	51.20 A	15.66 a
4.00	33.32 b	12.98 b
8.00	15.76 c	9.27 c
F. test	**	0.55
Interaction	**	**

Table 3. Mean of transpiration rate (mol h2O. m^{-2} s^{-1})and stomatal conductivity as (mol m^{-2} s^{-1})influenced by various varieties and salt levels

Trait / Treatments	Transpiration rate	Stomatal conductivity
Varieties		
Giza 177	7.22 c	0.347 b
Giza 178	9.17 a	0.447 a
M 201	8.07 b	0.364 b
F. test	**	**
Salt levels dS/m :		
0.98	9.52 a	0.491 a
4.00	8.36 b	0.398 b
8.00	6.51 c	0.262 c
F. test	**	**
Interaction	NS	NS

Table 4. Mean of air to leaf water vapor pressure deficit (Kpa) and relative chlorophyll content as influenced by various varieties and salt levels

Trait / Treatments	Air to leaf water vapor pressure deficit	Relative chlorophyll (SPAD)
Varieties :		
Giza 177	2.38 a	40.38 b
Giza 178	2.20 b	34.86 c
M 201	2.38 a	44.49 a
LSD 0.05	**	**
Salt levels ds/m :		
0.98	2.10 c	41.74 a
4.00	2.26 b	4.077 a
8.00	2.60 a	37.22 b
F. test	**	-
Interaction	NS	**

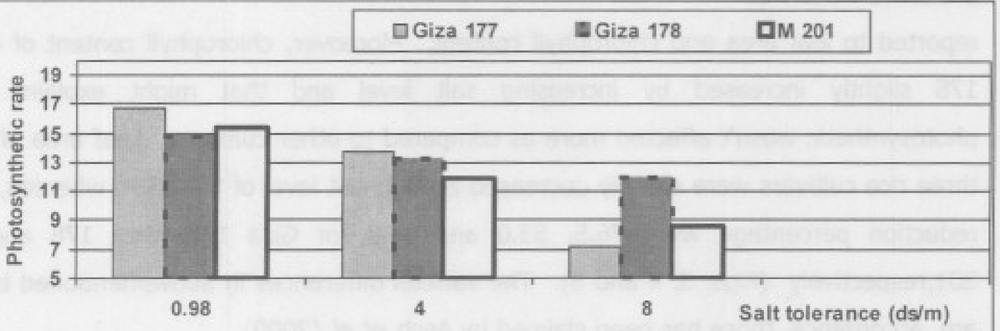

Fig. 3. Mean of photosynthetic value as affected by the interaction between rice cultivars and salt levels

Interaction effect

From the obtained data, the interaction between rice cultivars and salt levels had significant effect on photosynthetic rate, leaf area and chlorophyll content. It was obvious that the photosynthetic rate of sensitive cultivars Giza 177 and M 201 were dramatically reduced under higher salt levels (reduction percentage at 8.0 dS/m were 57.3 and 44.3% for Giza 177 and M 201, respectively).

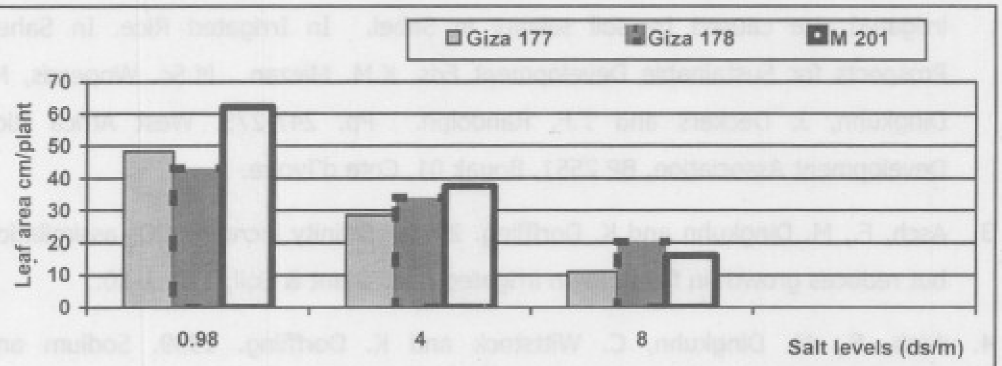

Fig. 4. Mean of leaf area/plant as affected by the interaction between rice cultivars and salt levels

Whilst, Giza 178 had the ability to keep less reduction in its photosynthetic rate (18.9 at 8.0 ds/m). It is worth to mention here that

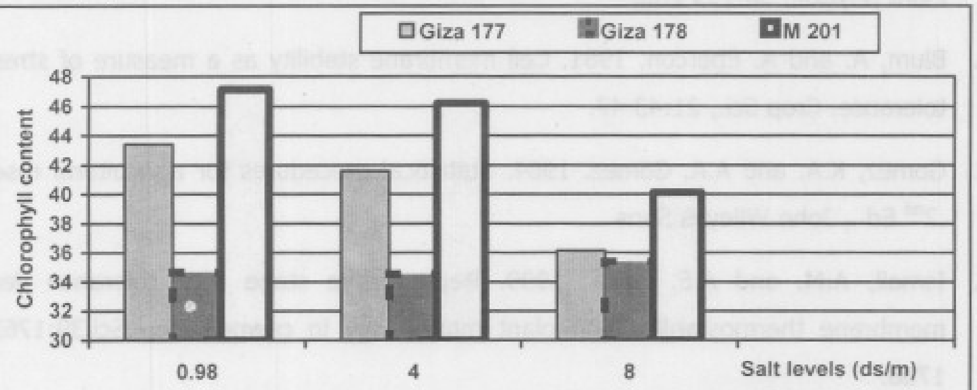

Fig. 5. Mean of relative chlorophyll content as affected by the interaction between rice cultivars and salt levels

the same trend was
reported to leaf area and chlorophyll content. Moreover, chlorophyll content of Giza 178 slightly increased by increasing salt level and that might explain why photosynthetic wasn't affected more as compared to other cultivars. Leaf area of the three rice cultivars were sharply decreased at high salt level of 8.0 dS/m whereas, the reduction percentage were 76.5, 53.0 and 74.3 for Giza 177, Giza 178 and M 201,respectively (Figs. 3, 4 and 5). The varietal differences in abovementioned traits are accordance those has been claimed by Asch *et al.* (2000).

REFERENCES

1. Agarie, S., N. Hanaoka, F. Kubota, W. Agat and P.B. Kaufman 1995. Measurement of cell membrane stability evaluated by electrolyte leakage as drought and heat tolerance test in rice. J. Fac. Agr. Kyushu Univ., 40(1-2): 233-240.
2. Asch, F., M. Dingkuhn and K. Dorffling. 1997. A physiological stresses of irrigated rice caused by soil salinity in Sahel. In Irrigated Rice. In Sahel: Prospects for Sustainable Development Eds. K.M. Miezan. M.Sc. Wopercis, M. Dingkuhn, J. Deckers and T.F. Randolph. Pp. 247-273. West Africa Rice Development Association, BP 2551, Bouak 01, Cote d'Ivoire.
3. Asch, F., M. Dingkuhn and K. Dorffling. 2000. Salinity increase CO_2 assimilation but reduces growth in field grown irrigated rice. Plant & Soil, 218: 1-10.
4. Asch, F., M. Dingkuhn, C. Wittstock and K. Dorffling. 1999. Sodium and potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. Plant Soil. 207, 133-145.
5. Bewley, J.D. 1979. Physiological aspects of desiccation tolerance. Annu. Rev. Plant phyisol., 30:195-238.
6. Blum, A. and A. Ebercon. 1981. Cell membrane stability as a measure of stress tolerance. Crop Sci., 21:43-47.
7. Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural roses .2nd Ed ., John Wiley & Sons .
8. Ismail, A.M. and A.E. Hall. 1999. Reproductive stage heat tolerance ,leaf membrane thermostability and plant morphology in cowpea.Crop Sci.39:1762-1768.

9. Mckersie, B.D. and T. Tomes. 1980. Effect of dehydration treatment on germination, vigor and ctytoplasmic leakage in wild oats and birdsfoot trefoil. Can. J. Bot., 58:471-476.

10. Mckersie, B.D., P. Hul and W.D. Beversdrof. 1982. Solute leakage from susceptible cultivars of *Phaseolus.vulgars* following ozone exposure. Can J. Bat., 60:73-78.

11. Premachandara, G.S., H. Saneoka, and S. Ogata. 1989. Nutrio-physiological evaluation of polyethylene glycol of cell membrane stability in maize. Crop Sci., 29: 1287-1292.

12. Premachandra, G.S. and T. Shimada. 1987. Measurement of drought tolerance in orchardgrass (*Dactylis glomerata* L.). Polyethylene glycol test of measuring cell membrane stability. J. Japan Grassl Sci.,33:140-148.

13. Sultana, N., T. Ikeda and R. Itoh. 1999. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environmental and Experimental Botany. 42: 211-220.

14. Yoshida S.A., F. Douglas, H.C. James and A.G. Kwanchai. 1976. Laboratory manual for physiological studies of rice. IRRI press. Manila, Philippine.

قدرة ثبات الغشاء الخلوي ، معدل البناء الضوئي ومكوناته كدليل على تحمل الملوحة فى بعض أصناف الأرز

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فى هذه الدراسة كان الهدف محاولة دراسة صفة الغشاء الخلوي في بعض أصناف الأرز النامية تحت ظروف الملوحة كمؤشر لتحمل الملوحة وكذلك معدل البناء الضوئي ومكوناته .

وبناءا على ماتقدم تم اختيار ثلاثة أصناف مختلفة فى تحملها للملوحة ، جيزة ١٧٧ (صنف مصري حساس للملوحة) جيزة ١٧٨ (مصري متحمل الملوحة) ، أم – ٢٠١ (صنف أمريكي حساس) نمت تحت ثلاثة مستويات من الملوحة هي ٩٨ ، ٤,٠ ، ٨,٠ ملليموز /سم . وأقيمت التجربة فى ستة مكررات بتصميم القطع المنشقة حيث احتلت الأصناف القطع الرئيسية ومستويات الملوحة القطع المنشقة أقيمت التجربة بمعمل الملوحة الأمريكى ريفر سايد – كاليفورنيا – الولايات المتحدة الأمريكية – عام ٢٠٠٠.

معدل ثبات الغشاء الخلوي ومعدل البناء الضوئي والتوصيل الثغري ومساحة الورقة وكذلك نقص الضغط البخاري المائي من الهواء إلى الوراثة وكذلك المحتوى الكلوروفيل قد تم تعزيزه عند ٤٠ يوم من الزراعة.

ومن النتائج أتضح أن أكثر الأصناف قدرة على الحفاظ على معدل ثبات الخلية وكذلك إظهار أقل نقص تحت ظروف الملوحة فى معدل البناء الضوئي والتوصيل الثغري وبقية الصفات هو الصنف المصري المتحمل للملوحة جيزة ١٧٨ بينما أظهر كل من الصنف جيزة ١٧٧ ، أم – ٢٠١ حساسية عالية للملوحة حيث أظهر نقص شديد فى مدى ثبات الغشاء الخلوي وكذلك معدل البناء الضوئي وبقية مكونات عمليات البناء الضوئي فأعلى نسبة تسرب الكتروليتات كانت بواسطة الصنف جيزة ١٧٧ تلاه الصنف الأمريكي أم – ٢٠١ ومن ناحية أخرى وجد أن زيادة الملوحة تقلل من ثبات الغشاء الخلوي وتزيد من التسرب الإلكتروني وكذلك تقلل من عملية البناء الضوئي ومسام الورقة بشدة وكذا التوصيل الثغري بينما يزيد من نقص الضغط البخاري من الهواء إلى الورقة ووجد أن كلوروفيل الصنف جيزة ١٧٨ لم يتأثر مطلقا بالملوحة . وكان هناك تأثير معنوي من جانب التفاعلات على معدل ثبات الغشاء الخلوي ، مساحة الورقة البناء الضوئي والكلوروفيل.