STUDY ON THE IRRADIATED RICE MANAW THUKHA (MASHURI-M) FOR THE SALINITY TOLERANCE AT VEGETATIVE AND REPRODUCTIVE STAGES

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Abstract - Nowadays, the whole world is facing climate changes such as flood, salinity and drought. Crops cultivated in central Myanmar are also badly affected by such climate changes. This fact necessitates to develop crop varieties that can tolerate environmental changes. In this study, Manawthukha var. irradiated by Gamma irradiation, which can tolerate salinity, is chosen. Screening under field conditions is difficult due to stress heterogeneity, presence of other soil-related stresses, and the significant influence of environmental factors such as temperature, relative humidity, and solar radiation. Saline soils are usually under waterlogged condition; other crops could not grow in these areas except rice. Dry seeds of Manawthukha were exposed to gamma radiation ranging from 0Gy to 400Gy to determine their responses to radiation and the effective doses for mutation breeding. The M1 seeds of this variety were sown in the field along with the control (non-irradiated seeds) and then, they were harvested. The main panicles from M1 were sown in the laboratory screening for M2 generation. In M3 generation, it was observed that plant height, panicle length and 1000 grains weight of some selected mutant lines were higher than that of the control plants. Reducing yield per hill affected by the late flowering can be seen in MK-C mutant lines. The experiment of hydroponic screening with the dose of 400 Gy was tolerant than other gray at seedling stage in M_2 generation. However, filled grains per panicle and yield per hill of M1 were generally decreased with the increasing doses of gamma ray. In M4 generation, it was observed that MK-A, MK-A1, MK-B1, MK-C1, MK-D2, MK-F, MK-F2, and MK-F4 mutant lines and control seeds became sterile under saline condition though the heading was appeared.

Keywords - Manawthukha, Gamma Irradiation, Mutation, Salinity Tolerance, Vegetative stage, Reproductive srtage.

I. INTRODUCTION

Rice is one of the most widely cultivated crops which provide food for one-half of the world's population [1]. The soil salinity of reclaimed paddy fields is one of the important stresses which limit rice growth and yield in Asia and Africa [2]. There are 380 million ha of saline soils on the earth's land surface which are widely distributed in arid and semi-arid areas and seasonally dry coastal areas, where they severely affect the agricultural production of many countries [3]. Myanmar produce two rice crops annually: wet season and dry season. Wet season production typically accounts for 85% of total production, while the dry season the remaining 15%. Myanmar's paddy fields can be found mostly in the delta and central dry zone areas. The drought area of rice cultivated in Myanmar is 12% about 155140 hectares. Some 14% (173560 hectares) of rice area are affected by submergence in Myanmar. The total rice crop are affected by salinity is about 3% (513780 hectares) in Myanmar. Rice is sensitive to salinity particularly at the seedling stage. Screening/breeding of rice varieties for tolerance to salinity have been carried out for over three decades and various screening methodologies are used to screen out tolerant varieties [4]. Although salinity affects all stages of growth and development of rice, salinity at the reproductive stage depresses grain yield much more than salinity at the vegetative stages, therefore, screening for tolerance at reproductive stages has been considered to be more useful [5]. The use of physiological characters as selection criteria in salt tolerance breeding requires the identification of the contribution of each individual character to salt tolerance [6]. Plant growth, plant height or shoot biomass was reported to have dilution effects on sodium accumulation in leaves of rice. Panicle weight, tiller numbers per plant and harvest index are important agronomic characters for the prediction of final yield in rice. These yield components are severely affected by salinity [7].

Saline soils are one of the major problems in agriculture, limiting crop growth and production in many parts of the world. The impact of salinity on agricultural productivity has been examined. Rice is moderately sensitive to salinity [8]. Most rice is grown in tropical and sub-tropical lowlands and most commonly in soils submerged under water. Salinity, however, is an important toxicity encountered by rice. On the other hand, rice is relatively tolerant to salinity and is widely grown in tidal swamps and in inland areas frequently subject to salinity [9].

Salinity is the saltiness or dissolved salt content of a body of water. Salt are compounds like sodium chloride, magnesium sulfate, potassium nitrate, and sodium bicarbonate which dissolve into ions. Salinity is an ecological factor of consideration importance, influencing the types of organisms that live in a body of water. Salinity reduces photosynthesis rate, metabolism process, carbohydrate translocation, dry matter production, leaf area index, nutrient absorption, all yield attributes and rice grain yield, while it increases sterility percentage of rice [10].

Rice is sensitive to saline stress at the seedling stage and reproductive stage. Salinity tolerance in rice is complex and involves several contributing traits. Most of the varieties developed by breeders in self-pollinated crops like rice are based on single crosses between two parents. Breeders have also attempted to make multiple crosses to increase the genetic variation in breeding populations [11].

Salinity is one of the major obstacles in increasing in rice growing areas worldwide, which is an ever-present threat to crop yield. Several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage becomes sensitive during pollination and fertilization and then become increasingly more tolerant at maturity [12]. Screening of germplasms at seedling stage in readily acceptable as it is based on a simple criterion of selection; it provides rapid screening difficult at vegetative and reproductive stage [13].

Screening at field level proved to be difficult due to soil heterogeneity, climatic factors and other environmental factors which may influence the physiological processes. Hence, screening under laboratory conditions is considered to be advantageous over field screening. However, conventional methods of screening for salt tolerance are not easy because of environmental effects and narrow sense heritability of salt tolerance [14]. The conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance.

II. MATERIALS AND METHODS

A. Plant Material

Rice cultivar viz., Manawthukha (Mashuri-M) was used as the experimental material in this study. The life period of Manawthukha is about 135 days. Its original name is Mashuri M and supposedly originated in Malaysia. It is a semi-dwarf type with a plant height of 105 cm, 10 to 12 number of effective tillers, 19 g of 1000 grains weight and it has a droopy panicle axis.

B. Induction Treatment

Pure and dry seeds of Manawthukha (500 seeds) were treated with 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy and 400 Gy of gamma rays from the ^{60}Co at the Department of Atomic Energy (DAE), Kabaraye Pagoda Road, Yankin Township, Yangon. This experiment was carried out at the Department of Biotechnology, Technological University (Kyauk Se), Mandalay Division.

C. Preparation of Peter Solution and Salinization

Water soluble fertilizer as hydroponic solution was used by Peter's (20-20-20) at a rate of 1 g per L purified drinking water. 100 mg per L of ferrous sulphates ($FeSO_4$) were also added the solution was stirred. The pH of the solution was adjusted to pH 5.0-5.1 by using pH meter every two days. 1N NaOH and 1N HCL were used to adjust the pH of the solution. Different concentrations of NaCl were added to the nutrient solution (7.013 g NaCl per Liter of nutrient solution gives an EC 12 dS m^{-1}) measuring by Ec meter. Test entries were rated at 10 and 16 days after initial salinization.

D. Evaluation of rice genotypes at seedling stage

At seedling stage, germinated seeds were sown in hydroponic system with distil water in a plastic box (6 liters). Fifty seeds per line, on a Styrofoam sheet with 8 lines attached a nylon net bottom (see Fig. 3). The sheet was floated on the distilled water with Peter nutrient solution. A population of 8 types (7 rice cultivars) and 1 pokkili (tolerant check) were tested in salt stress (0, 6, 8, 10 and 12 dS m^{-1}).

E. Modified standard evaluation score (SES)

Scoring was done according to the modified standard evaluation system used in rating of the visual symptoms of salt toxicity injury [IRRI, 1997] (Table I).

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips of few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are Elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying.	Susceptible
9	Almost all plants dead or dying	Highly susceptible

TABLE I
Standard Evaluation System For Scoring Of Visual Salt Injury At Seedling And Reproductive Stage In rice

III. RESULTS AND DISCUSSION

A. M1 Studies

The M1 seeds of this variety were sown in the field of Technological University (Kyaukse) with the control (non-irradiated seeds), 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy and 400 Gy seeds that can be seen in figure 1 and some agronomic characters of M_1 generation can be seen in Table II. According to Table II, the filled grain of 400 Gy was higher than that of other treatments but the panicle length of this variety was the lowest. At maturity, the main panicles from this variety were harvested for M_2 generation.

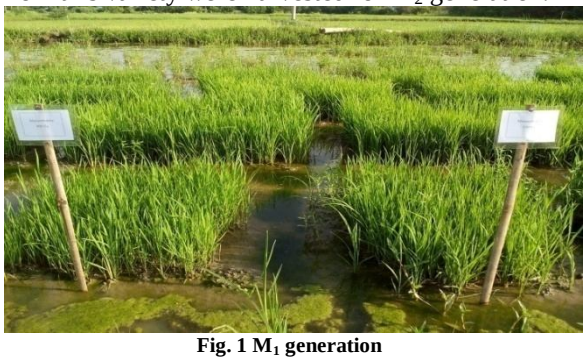


Fig. 1 M_1 generation

Treatment	Plant Height(cm)	Panicle Length (cm)	Effective Tillers	Filled Grain	1000 Grains Weight (g)
Control	89.69	22.45	12.62	90.689	21.8
100 Gy	90.5	22.37	13.07	95.1	19.96
150 Gy	87.1	21.73	10.73	88.9	20.53
200 Gy	87.72	21.79	11.59	90.517	20.25
250 Gy	84.17	22.32	13	78.467	20.31
300 Gy	85.76	21.41	10.34	72.586	19.86
350 Gy	90.47	23.6	9.2	77.133	21.5
400 Gy	89.5	21.3	11.67	111	21.72

TABLE II
MEAN VALUES OF SOME AGRONOMIC CHARACTERS FROM M_1 GENERATION IN SUMMER SEASON, 2015

B. M2 Studies

For M_2 generation, the main panicles of M_1 plants were collected and 19 plants from each treatment were screened by hydroponic screening method. So for seven treatments, a total of 136 plants, including control, IR29 and pokkali were screened. Among them only 62 plants survived. They were screened again for M_3 generation. Pokkali scored 3, while control scored 5. Among a total of 135 plants, 68 plants also scored 5, 6 plants scored 3 and 54 plants scored 7 which means 54 plants were susceptible. The different salinity level of M_2 generation was shown in Table II. The mean performance of yield components was listed in Table V. It can be observed that the plant height and 1000 grains weight of control was higher than that of all treatments. However, the effective tillers and panicle length of all treatments were decreased the increasing in the dose of radiation.

Name of the Variety	Salinity Level			
	EC 0	EC 6	EC 8	EC 12
MTK Control	1	1	3	3
Pokkali	1	1	1	3
IR-29	1	1	1	3
100-1	1	1	3	5
100-2	1	1	3	5
100-5	1	1	3	5
100-7	1	1	3	5
100-8	1	1	3	5
100-12	1	1	3	5
100-13	1	1	3	3
100-14	1	1	3	3
100-15	1	1	3	5
100-16	1	1	3	5
100-18	1	1	3	5
150-1	1	1	3	5
150-2	1	1	3	5
150-4	1	1	3	7
150-5	1	1	3	5
150-7	1	1	3	5
150-8	1	1	3	5
150-10	1	1	3	3
150-11	1	1	3	7
150-12	1	1	3	5
150-13	1	1	3	3
150-14	1	1	3	5
150-16	1	1	3	5
200-2	1	1	3	5
200-4	1	1	3	5
200-6	1	1	3	5
200-10	1	1	3	5
200-12	1	1	3	5
200-13	1	1	3	3

200-14	1	1	3	5
200-18	1	1	3	5
250-2	1	3	5	7
250-4	1	1	3	5
250-5	1	1	3	5
250-10	1	1	3	5
250-13	1	1	3	5
250-14	1	1	3	5
250-18	1	1	3	5
300-2	1	3	5	7
300-4	1	1	3	7
300-6	1	1	3	5
300-8	1	1	3	5
300-10	1	1	3	5
300-13	1	1	3	7
300-15	1	1	3	5
300-16	1	1	3	5
300-18	1	1	3	7
350-1	1	1	3	7
350-2	1	3	5	7
350-10	1	1	3	7
350-12	1	1	3	5
350-18	1	1	3	7
400-2	1	1	3	3
400-4	1	1	3	5
400-5	1	1	3	5
400-7	1	1	3	5
400-10	1	1	3	7
400-12	1	1	3	5
400-13	1	1	3	5
400-14	1	1	3	5
400-16	1	1	3	5
400-18	1	1	3	5

TABLE IIII
SALT TOLERANCE OF M2 GENERATION DURING SEEDING STAGE AT DIFFERENT SALINITY LEVEL



Fig. 2 Vegetative stage and Reproductive stage in M_2 generation

C. M3 Studies

Among 62 plants from M_2 generation, the plants which were not better than the control plants were not screened for M3 generation. Nineteen plants including pokkali, IR29 and control were selected and screened again for M3 generation and also they were regarded as the mutant lines and were cultivated in rainy season. The mean performance of yield components was listed in Table V and the different salinity level shown in Table III for M_3 generation. The plants in mutant lines MK-C1, MK-D1, and MK-F1 flowered earlier than the normal plants whereas the mutant lines MK- A, MK-A2, MK-B, MK-B1, MK-C, MK-E and MK-F2, MK-F3, and MK-F4 showed late flowering. It can be observed that the plant height of control was lower than the promising line of MK-D2 and not significantly lower than other mutant lines. Then MK-C1 had the lowest number of effective tillers and yield per hill. Although the panicle length in MK-D was significantly higher than that of the mutant plants and control, the number of tillers in control was significantly higher than that of the mutant lines. However, MK-F3 had the highest number of 1000 grains weight .The promising lines in MK-D2 and MK-E of yield per hill was significantly higher than that of the mutant plants.

Name of the Variety	Salinity Level			
	EC 0	EC 6	EC 10	EC 12
MTK Control	1	1	3	3
Pokkali	1	1	1	1
IR-29	1	1	1	1
MK-A	1	1	3	3
MK-A1	1	1	3	3
MK-A2	1	1	3	5
MK-B	1	1	3	5
MK-B1	1	1	3	3
MK-C	1	1	3	5
MK-C1	1	1	3	5
MK-D	1	1	3	5
MK-D1	1	1	3	5
MK-D2	1	1	3	5
MK-E	1	1	3	7
MK-F	1	1	3	3
MK-F1	1	1	3	5
MK-F2	1	1	3	5
MK-F3	1	1	3	5
MK-F4	1	1	3	7

TABLE IVV
SALT TOLERANCE OF M3 GENERATION DURING SEEDING STAGE AT DIFFERENT SALINITY LEVEL



Fig. 3 Vegetative stage and Reproductive stage in M_3 generation

D. Experimental set up for screening

In this experiment, all genotypes including checks were grown in normal condition and exhibited 100% survival in absence of salt stress. Different degrees of salt injuries (scores 3 to 9) and growth retardation was observed in all lines under salt stressed condition. The genotypes showed wide variation in comparison with (MTK 400-2) having score 3 and (MTK 300-2) having score 9 (see Figure 4). Gregorio et al. (2002) and Jubay (2012) [14] also observed wide variations in Pokalli (tolerant) and IR29 (sensitive).

E. Evaluation of salt stress symptoms

The modified standard evaluating score was used (see Figure 4) in rating the visual symptoms of salt toxicity. This scoring discriminates the susceptible from the tolerant and the moderately tolerant genotypes. Scoring started at 10 days after salinization and final scoring at 16 d after salinization. At 10 days after salinization, Pokkali scores: 1 (highly tolerant); MK-F: 1 (highly tolerant). During this time, susceptible genotypes could be distinguished from the test entries. However, tolerant types could not be readily identified from the moderate ones. At 16 d after salinization Pokkali scores: 3 (tolerant); MK-F: scores 3(tolerant). At that time, there was a clear distinction among the test entries: the tolerants, moderates,and the susceptibles.

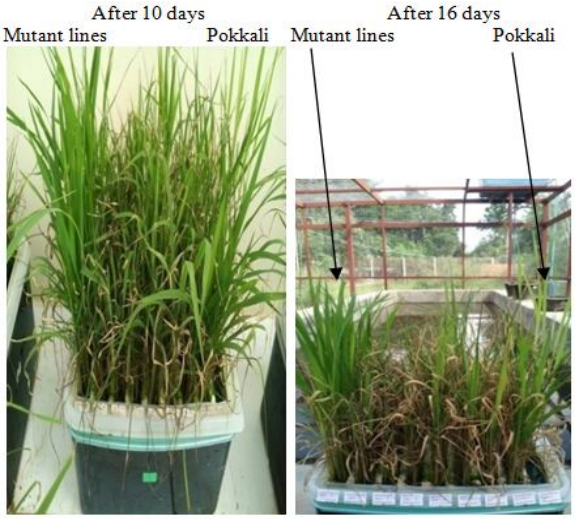


Fig.4 Mutant lines 0f MTK at EC 10 and 12.

Salt injury symptoms were shown after 7 to 8 days from the first day of salinization. The main symptoms were leaf rolling and formation of the new leaves, leaf rolling and whitening of the tips which lead to complete cessation of the growth and finally drying the leaves. Jubay (2012) and Islam (2004) [15] observed similar salt injury at the seedling stage of rice plant in Pokkali and IR29.

Treatment	Plant Height(cm)	Affective Tiller	Panicle Length (cm)	Filled Grain	1000 Grains Weight (g)
Control	105.86	14.68	23.59	103.27	21.8
100 Gy	106	14.15	23.25	115.8	19.96
150 Gy	103.9	11.05	21.65	107.15	20.53
200 Gy	95	10.05	21.87	100.47	20.25
250 Gy	91.45	12.05	21.95	106.6	20.31
300 Gy	95.7	9.87	22.9	87.95	19.86
350 Gy	95.8	10.5	22.9	85.4	21.5
400 Gy	94.7	7.8	22.3	62.3	21.72

TABLE V
MEAN VALUES OF SOME AGRONOMIC CHARACTERS OF POTENTIAL LINES SELECTED FROM M2 GENERATION IN RAINY SEASON, 2015

Potential Lines	Days to 50% flowering	Plant Height (cm)	Panicle Length (cm)	No. of Tillers	No. of Effective Tillers	1000 Grains Weight (g)	Yield per Hill (g)
MTK Control	124	75.2	20.2	20.6	10.8	19.98	2.26
MK-A	125	67.67	18.33 ns	11.67*	6 ns	16.67*	3.997 ns
MK-A1	120	64.67*	17.54*	9.22*	4.44*	15.68*	2.69 ns
MK-A2	127	70.75 ns	18.63 ns	12.75*	5.75*	15.75*	3.85 ns
MK-B	126	69.33 ns	21.67 ns	10.67*	5.67 ns	15.6*	3.72 ns
MK-B1	124	71.45 ns	20.8 ns	9.25*	2.95*	19.9 ns	3.09 ns
MK-C	137	60.5*	17.25*	9.5*	6.25 ns	15.18*	2.63 ns
MK-C1	119	68.67 ns	20.23 ns	9.33*	2.67*	15.47*	2.13 ns
MK-D	122	68 ns	22 ns	14.5 ns	4 ns	17.38	2.84 ns
MK D1	110	65.33*	19.33 ns	11.33*	5.67 ns	16.43*	4.93 ns
MK-D2	100	76.85 ns	19.26 ns	14*	12.42 ns	18.37 ns	5.31*
MK-E	129	72.5 ns	20.5 ns	10.5*	6 ns	18.37 ns	5.44 ns
MK-F	120	59.4*	17.21*	9.2*	6.87*	15.06*	2.96 ns
MK-F1	118	59*	18.5 ns	9.25*	5.75*	18.12 ns	2.73 ns
MK-F2	125	67 ns	19.033 ns	7.67*	3.67*	16.5*	4.13 ns
MK-F3	124	70.2 ns	17.64 ns	12.6*	10.8 ns	20.05 ns	2.61 ns
MK-F4	124	60.6*	17.4*	11.4*	7.2 ns	18.9 ns	2.8 ns

TABLE VI
MEAN VALUES OF SOME AGRONOMIC CHARACTERS OF POTENTIAL LINES SELECTED FROM M3 GENERATION IN RAINY SEASON, 2016

CONCLUSION

In this study, it was observed that salinity tolerance at the seedling stage showed wide variation in phenotypes with salinity scores ranging from 3 to 9. Among the promising lines, (MK-A, MK-A1, MK-B2 and MK-F) mutant line were found tolerant at EC 12 dS m^{-1}, (MK-A, MK-A2, MK-B, MK-B1, MK-F1, MK-F2, MK-F3, MK-F4) resistance lines were found moderated tolerant at EC 12 dS m^{-1} and (MK-A1, MK-B2, MK-C, MK-C1, MK-D, MK-D1, MK-D2, MK-E) were sensitive to salt stress at EC 12 dS m^{-1}. Comparison with the irradiated mutant lines, the non-irradiated control ones were completely under salt affected although they can reach to heading stage. The promising and salt stress tolerant mutant lines will be cultivated in next generation to confirm if they can be tolerant to salt stress and then the Primary Yield Trial of these mutant lines will be tested.

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