

Development of Drought Tolerant Mutant from Rice var. Manawthukha through Mutation Breeding Technique using ^{60}Co Gamma Source

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ABSTRACT: Water deficit reduces plant growth and development, leading to the production of lower yield. This study was focused on determining the effects of water stress on promising mutant lines of rice var. Manawthukha. Dry seeds of rice, Manawthukha were irradiated with the dose of 300 Gy of gamma rays from ^{60}Co source. The treatment of water exclusion was started to the plants from 90 days after sowing (DAS) throughout reproductive stage until harvesting time. A selection process was made in M_2 generation on the basis of agronomic traits. The selected promising lines in M_2 were evaluated against non-irradiated control plants in M_3 generation. The desiccation of apical spikelets in stressed panicles was obviously found in summer cultivation in M_4 generation. Hence, the control plants were not fertile under water deficit condition while other mutant plants are fertile at that time. The two best mutant lines namely MK-D-2 and MK-D-3 were selected in M_6 generation and characterized by using physiological screening techniques such as relative water content (RWC), soil moisture content and yield. In comparison with the well-watered condition, the mutant lines cultivated in drought stress condition produced less grain. The relative water content of mutant plants was between 91% and 93% while those of control plants were 83%. Therefore, it was observed that the mutant plants are able to uptake more water than control plants in water stress condition.

KEYWORDS: Agronomic traits, Drought stress, Gamma radiation, Relative water content, Soil moisture content.

I. INTRODUCTION

Rice, in Myanmar, is one of the most important crops, grown on 6.0 million hectare or about two-thirds of the country's total cultivated area. Like other countries, Myanmar also suffers from climate change impacts on water cycle by sudden change of weather pattern such as flood and long drought and thus sustainability of water environment in some areas are facing difficulties. In rainfed ecosystems, drought is a major limitation for rice production. Due to the climate change impacts rainfall pattern and rainfall intensity are significantly changed occasionally in some parts of the country depending on the topographical condition. In the middle part of Myanmar, especially in dry zone area, sometime intervals of non-rainy days last more than two or three weeks and annual rainfall intensity is rather less than normal average. As a consequence, lesser inflows into the reservoirs, resulting in irrigation water shortage problems particularly in dry season.

Drought stress can lower the ability of roots to absorb enough water which leads to wilting. This can reduce the photosynthetic rate as well as the uptake of minerals from the soil. The stressed plants usually slow or stop growth and flowering, abort grain and drop leaves. Furthermore, it has been reported that the reproductive developments in grain crops was more sensitive to water stress than the vegetative development. In other words, the presence of water stress during plant reproduction stage is detrimental to the reproductive organ [1].

Breeding of modern varieties has played an important role in agriculture and increasing the income for farmers. Mutation breeding has played a significant role in the last 75 years by releasing around 2672 mutant varieties for

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 11, November 2015

commercial cultivation in the world. Mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars. Officially released mutation-derived varieties such as rice, wheat, cotton and sunflower have made a major economic impact in many countries [2]. The use of induced mutation in crop improvement has proven to be an effective approach to improve yield, quality and resistance to biotic and abiotic stresses [3].

This study was intended to produce high yielding mutant varieties with desired drought tolerant characters caused by the climate change through induced mutation and their impact on growth of rice under stress environment.

II. MATERIALS AND METHODS

A. Plant Material Used

Plant material used in this investigation was the local rice variety Manawthukha. This variety is widely grown cultivar in Myanmar because it is photoperiod insensitive and can grow in both rainy and summer seasons. The main feature of this is its medium maturity and high-yield potential. Dry seeds of rice, Manawthukha were irradiated with the dose of 300 Gy of gamma rays from ^{60}Co source at the Department of Atomic Energy (DAE), Ministry of Science and Technology (MOST), Yangon.

B. Experimentation

The climate in Kyauk-Se is semi-arid and by large seasonal variations for both rainfall and temperature. The average monthly temperature ranges from 21°C to 37°C during 2013 and 2014. The total rainfall recorded during the growth cycle was 115 mm and 87 mm in the 2013 and 2014 respectively. The months of April, May, June and July have the highest temperatures (35-39°C). Rainfall occurs mainly in September-October and the rainy days are very short. Daily weather parameters (precipitation and air temperature) were collected from the weather station located near the trials. The soil in experimental field was silty loam in texture having pH 8.1.

The treatment of water stress was exposed to the plants from 90 days after sowing (DAS) throughout reproductive stage until harvesting time. Rice growth patterns registered by national and international agricultural research centers in Myanmar were followed. Fertilizer application was 120 kg/ha of urea and 250 kg/ha of N, PO_5 , K_2O compound. Field-managed stress screening was done under both wet season and dry season. Drought screening can be influenced by the effects of both wet and dry seasons. The wet season screening can have the influencing factors of fluctuating temperatures and high humidity, though it represent similar conditions as prevalent in the farmers field during the main rice growing season. The dry season which permits easy establishment of a controlled drought situation can be influenced by high temperatures or heat stress.

C. Data Collection

Both irradiated and non-irradiated control seeds were sown in the experimental fields following Randomized complete block design (RCBD) with three replications, at a spacing of 20cm x 20cm, to raise the M_1 generation. At maturity, main panicle in each plant was bulk harvested. The seeds of M_1 plants were grown with single plant per hill during the summer as M_2 generation. Selection was carried out in M_2 generation for traits of agronomic significance such as (plant height, panicle length, effective tiller, filled grains per panicle, thousand grains weight and yield per hill) under water stress condition. The selected mutants were confirmed in M_3 generation and those showing phenotypic stability were evaluated in M_4 generation. Further evaluation was carried out in M_5 and M_6 generation. Finally, the data were statistically analysed by student's t-test through Microsoft Excel software.

D. Measurement of Soil Moisture Content

Soil moisture measurement was carried out in M_6 generation by digital soil moisture meter (Model MO750) at the different soil depths (10cm, 20cm and 30cm) during the rice growing period. The first measurement was started 80 days after sowing (DAS) before the water stress to the plants. The second and last times measurements were done after the water stress imposing. Due to the large variation of measurements within each plot as the result of substantial heterogeneity within soils, 10 measurements were taken per plot and the average values were considered.

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E. Measurement of Relative Water Content (RWC)

The degree of drought stress was physiologically estimated by measuring the leaf relative water content (RWC) [4]. Leaf water content is a useful indicator of plant water status, since it expresses the relative amount of water present on the plant tissues. Leaf tissue is most commonly used for RWC determination. All components of leaf water relations change during the day as irradiance and temperatures change but for no more than two hours at and after solar noon, the change is very small [5]. Hence, the leaf samples were collected 7 days after water exclusion to the plants. The fully expanded leaf from mutant lines and control plants were taken from each plot in the afternoon between 1:00 pm and 2:00 pm. Each sample is placed in a pre-weighed airtight vial. The collected leaf samples are weighed to obtain the fresh weight of leaf sample, after which the samples are immediately hydrated to full turgidity for 12 hours under room light and temperature. Following surface drying with filter paper and turgid weight determination, the leaves are oven-dried at 80°C for overnight and reweighed to determine dry weight. The relative water content of a plant tissue is calculated according to the following formula [6]:

$$\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \times 100$$

Relative water content is a ratio of the amount of water in the leaf tissue a sampling to that present when fully turgid.

III. RESULTS AND DISCUSSION

A. Observation on Yield Component Characters through the Experiment

In M₂ generation, it was observed that the control (non-irradiated) plants were not fertile while some promising lines have completely matured even under the water deficit condition. The life period of rice var. Manawthukha is 135 days but the selected mutant lines were earlier flowering ten days than the control plants under water limited condition. Escape from drought is attained through a shortened life cycle or growing season, allowing plants to reproduce before the environment becomes dry. Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape [7].

From M₂ population, desirable seven mutant lines under drought stress condition were isolated on the basis of agronomic traits. The selected promising lines were evaluated against non-irradiated control plants in M₃ generation. M₃ generation was cultivated during July to December in rainy season. In this generation, some of mutant lines were flowered within 12 days after treatment earlier than the control plants. It is reported that flowering is slowed mainly due to slower elongation of the panicle and supporting tissues, with the onset of stress occurring from 10 to 5 days before heading [8]. The environmental condition such as raining makes the plants to impose mild stress at reproductive stage at field level experiment in rainy season and hence, it can be observed that there was no significant difference in yield component characters between the potential mutant lines, namely (MK-D, MK-E, and MK-F) and the control plants.

However, it has been reported that water stress significantly affected on the plant height [9]. The plant heights of potential three mutant lines under stress were found significantly higher than that of the control plants. Therefore, it was observed that the water stress significantly affected on the plant height of non-irradiated control plants rather than that of promising mutant lines. The mean performance of yield components in drought tolerant promising mutant lines and control plants was presented in "Table I".

TABLE.I. YIELD COMPONENTS OF DROUGHT TOLERANT POTENTIAL LINES FROM M₃ GENERATION IN RAINY SEASON, 2013

Potential Lines	Days to 50 % flowering	Plant Height (cm)	Effective Tiller	Panicle Length (cm)	Filled Grains/ Panicle	1000 Grains Weight (g)	Yield /Hill (g)
MK-control	112	84.15 ± 7.26	14.8 ± 3.49	18.87 ± 1.08	64.25 ± 31.05	18.3 ± 0.06	16.09 ± 5.92
MK-D	100	91.43 ± 6.61*	11.35 ± 3.18*	19.51 ± 1.3 ns	70.41 ± 16.87 ns	19.87 ± 0.74 ns	15.54 ± 4.6 ns
MK-E	100	94.35 ± 5.97*	11.65 ± 2.99*	20.99 ± 0.94*	66.26 ± 20.11 ns	21.6 ± 0.81 ns	15.96 ± 4.02 ns
MK-F	112	91.75 ± 7.19*	10.8 ± 3.64*	20.52 ± 1.37*	60.48 ± 17.02 ns	21.72 ± 0.49 ns	13.96 ± 5.34 ns

*= significant at 5% level ns= non-significant at 5% level
Each value represents the mean ± standard deviation (SD) of four replications.

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M₄ generation was sown from March to July in summer season, 2014. Of all the mutant lines, MK- D and MK- F were observed to fertilize at the maturing time. These lines produced seed before reaching the permanent wilting stage and although the plants did not recover after the stress period, viable seeds were produced, thus, ensuring the next generation. Although MK- E and control plants had reached to the flowering stage, they could not be fertile and so the spikelet sterility was occurred at maturing time. The flowering phase is one of the most sensitive periods to water stress in rice [10] and environmental stresses can cause a decline in spikelet fertility [11]. Therefore, the filled grains and 1000 grains weights in each potential mutant lines were about 30 to 40 % lower than that of the parent variety, leading to the lower yield per hill "Table. II". Furthermore, it has been reported that the spikelet sterility increases under water stress condition which might be due to the reduction of many key metabolic functions and physiological processes in plants [12].

TABLE.II. YIELD COMPONENTS OF DROUGHT TOLERANT POTENTIAL LINES FROM M₄ GENERATION IN SUMMER SEASON, 2014

Potential Lines	Days to 50 % flowering	Plant Height (cm)	Effective Tiller	Panicle Length (cm)	Filled Grains/ Panicle	1000 Grains Weight (g)	Yield /Hill (g)
MK-D-2	102	96.12	9.85	22.1	58.39	17.61	9.93
MK-D-3	102	89.63	8.53	22.83	42.32	19.4	6.6
MK-F-1	102	84.57	6.4	22.59	39.04	20.4	4.68
MK-F-2	102	91.34	10.53	24.1	60.45	20.53	13.27

Most studies also showed that spikelet fertility is sensitive to falling water status. The timing of the stress relative to the developmental stage of the crop has a large effect on spikelet fertility [13]. Promising mutant lines selected from M₄ generation, namely MK-D-2, MK-D-3, MK-F-1 and MK-F-2 with significantly improved drought tolerance and yield potential were grown as M₅ generation.

M₅ generation was cultivated from the month of August to December in monsoon season. Although the water exclusion was started in 90 days after sowing, before flowering, the rainy days in this season are during October which is the water stress treatment time in the experiment. However, it took two weeks water deficit to the plants in the field. This condition has been reported that withholding water for about 14 days from 10 days before flowering until 4 days after flowering can reduce yield by 20–40% relative to the fully irrigated aerobic control treatment [14]. This period was targeted because a relatively mild stress at this stage has a large impact on grain yield. From the experimental results in "Table III", it can be seen that the yield of control plants reduced significantly in comparison with three mutant lines (MK-D-2, MK-D-3 and MK-F-2) under water stress condition. The effect of anthesis date on drought response was associated with escape from drought. Therefore, the mutant plants were occurred flowering earlier than the control ones.

TABLE.III. YIELD COMPONENTS OF DROUGHT TOLERANT POTENTIAL LINES FROM M₅ GENERATION IN RAINY SEASON, 2014

Potential Lines	Days to 50 % flowering (DAS)	Plant Height (cm)	Effective Tiller	Panicle Length (cm)	Filled Grains/ Panicle	1000 Grains Weight (g)	Yield /Hill (g)
MK-control	112	93.88 ± 9.11	11.9 ± 2.13	18.5 ± 0.87	72.08 ± 14.9	19.1 ± 0.5	15.93 ± 1.16
MK-D-2	98	105.94 ± 1.6*	10.98 ± 2.24 ns	20.9 ± 0.5*	84.84 ± 6.55 ns	20.88 ± 0.1*	19.52 ± 4.86 *
MK-D-3	98	106.26 ± 4.94*	11.13 ± 0.89 ns	20.94 ± 0.36*	75.27 ± 18.12ns	21.75 ± 0.62*	17.99 ± 3.04 *
MK-F-1	98	98.04 ± 3.01 ns	9.1 ± 0.36*	20.85 ± 0.94*	94.82 ± 19.67 ns	21.83 ± 1.58*	15.77 ± 2.4 ns
MK-F-2	98	89.85 ± 7.44 ns	11.73 ± 0.47 ns	21.34 ± 0.66*	67.99 ± 8.55 ns	22.65 ± 1.99*	17.93 ± 1.27 *

*= significant at 5% level

ns= non-significant at 5% level

Each value represents the mean ± standard deviation (SD) of four replication.

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B. Observation on Yield Component Characters in M_6 Generation

The two best mutant lines namely MK-D-2 and MK-D-3 were selected in M_6 generation and these lines were characterized by using physiological screening techniques such as relative water content (RWC), soil moisture content and yield. M_6 generation was cultivated in summer season. In this generation, the yield component characters of mutant lines from both drought stress condition and irrigated condition were compared to know the severity of drought. Under well-watered condition, the mutant lines were earlier flowering 12 days than control plants.

Finding shows that the mutant lines yield significantly more grains than MK-control in stress condition. The mutant lines cultivated in drought stress condition produced from 20 to 40% less grain compared to well-watered condition “Fig. 1”. Poor panicle exertion is frequently observed with stress in the field, and thus it is clear that spikelet on the unexerted portion of the panicle remain unfilled. The stage of pollen meiosis has been suggested as a very drought sensitive stage. This stage occurs about 10 days before anthesis, and stress at that time is expected to reduce the number of normal pollen grains.

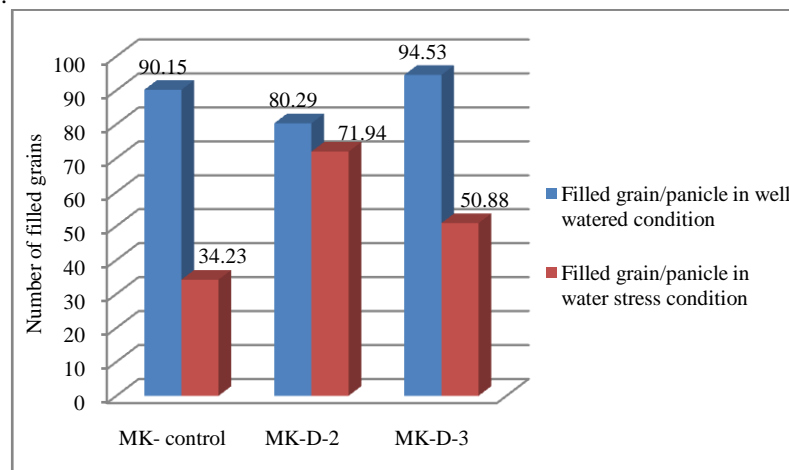


Fig. 1. Effect of well-watered condition and water stress condition on filled grain/ panicle of MK-control and mutant lines (MK-D-2 and MK-D-3)

Drought-induced yield reduction in rice depends upon the severity and duration of the stress period. In comparison with well-watered condition, it is still occurring yield reduction of mutant plants about 30% to 50% “Fig. 2”. It has been reported that a relatively mild stress and severe stress at reproductive stage has a large impact on grain yield and then mild stress at reproductive stage can reduce yield to 53-92% and severe stress at this stage reduce yield by 48%-94% [15].

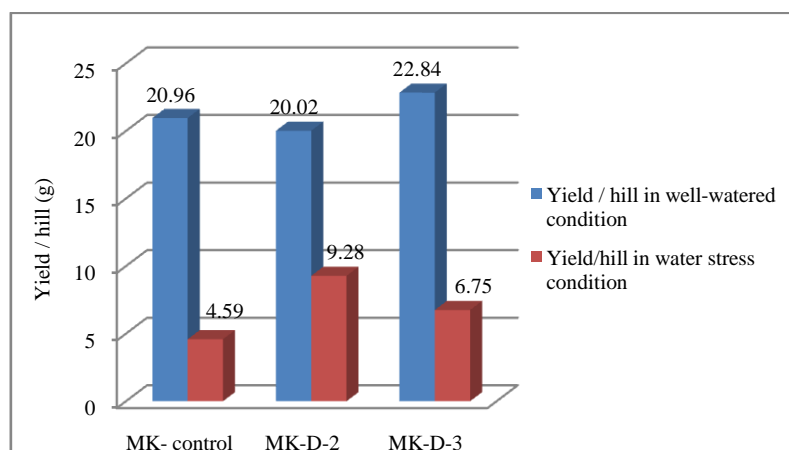


Fig. 2. Effect of well-watered condition and water stress condition on yield/hill of MK-control and mutant lines (MK-D-2 and MK-D-3)

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In well-watered condition, the mutant lines were about 12 days earlier mature than the MK-control though there were no significantly different effective tiller and yield per hill between the mutant plants and control "Table IV". Therefore, it was observed that the yield component characters of non-irradiated control plants and mutant lines were not different under no stress condition. In water stress condition, the number of filled grains per panicle in mutant lines was found higher significantly than in control "Table V". It has been reported that water stress reduced the number of grains per panicle [16].

TABLE.IV. COMPARATIVE PERFORMANCE OF DROUGHT TOLERANT MUTANTS AND CONTROL ON YIELD COMPONENTS IN WELL-WATERED CONDITION FROM M₆ GENERATION, 2015

Potential Lines	Mature Time (DAS)	Plant Height (cm)	Effective Tiller	Panicle Length (cm)	Filled Grains/ Panicle	1000 Grains Weight (g)	Yield /Hill (g)
MK-control	148	91.48 ± 5.01	13.5 ± 3.52	20.14 ± 0.95	90.15 ± 12.32	17.8 ± 0.31	20.96 ± 8.62
MK-D-2	132	87.85 ± 6.72 *	13 ± 3.51 ns	21.24 ± 1.71 *	80.29 ± 19.03 *	19.13 ± 0.03 *	20.02 ± 7.1 ns
MK-D-3	132	95.59 ± 2.73 *	13 ± 1.69 ns	22.44 ± 1.07 *	94.53 ± 28.35 ns	19.38 ± 0.4 *	22.84 ± 6.57 ns

*= significant at 5% level ns= non-significant at 5% level

Each value represents the mean ± standard deviation (SD) of four replications.

TABLE.V. COMPARATIVE PERFORMANCE OF DROUGHT TOLERANT MUTANTS AND CONTROL ON YIELD COMPONENTS IN WATER STRESS CONDITION FROM M₆ GENERATION, 2015

Potential Lines	Mature Time (DAS)	Plant Height (cm)	Effective Tiller	Panicle Length (cm)	Filled Grains/ Panicle	1000 Grains Weight (g)	Yield /Hill (g)
MK-control	158	92.37 ± 7.25	8.65 ± 3.77	19.97 ± 1.57	15.42 ± 0.2	34.23 ± 11.22	4.59 ± 2.74
MK-D-2	142	93.45 ± 6.42 ns	7.8 ± 4.15 ns	22.30 ± 0.8*	18.99 ± 0.1*	71.94 ± 29.54*	9.28 ± 4.05*
MK-D-3	142	101.5 ± 4.11*	9.25 ± 3.9 ns	23.05 ± 0.88*	19.92 ± 0.15*	50.88 ± 21.99*	6.75 ± 4.88*

*= significant at 5% level ns= non-significant at 5% level

Each value represents the mean ± standard deviation (SD) of four replications.

C. Observation on Physiological Screening Techniques in M₆ generation

The measured average values of relative water content on leaf samples of mutant plants under water limited condition were around 90% and it is about 10% higher than that of control plants having RWC 83%. Unstressed leaves will have a RWC of 90-95% depending on the humidity and light. Stressed and wilted leaves may drop as low as 50% [17]. Sustained plant water status is the primary reason for sustained function under drought. Since plant water status has a major impact on plant function and adaptive processes in plants under stress, the factors that affect plant water status under stress are the most crucial for drought tolerance. After 2 weeks without water the plants started to react visibly to the drought stress condition. Some plants started to lose chlorophyll in their lower leaves, while others inclined their leaves away from the sun. Therefore, it can be observed that the mutant plants were less sensitive to water shortage than control plants in water limited condition "Fig. 3".

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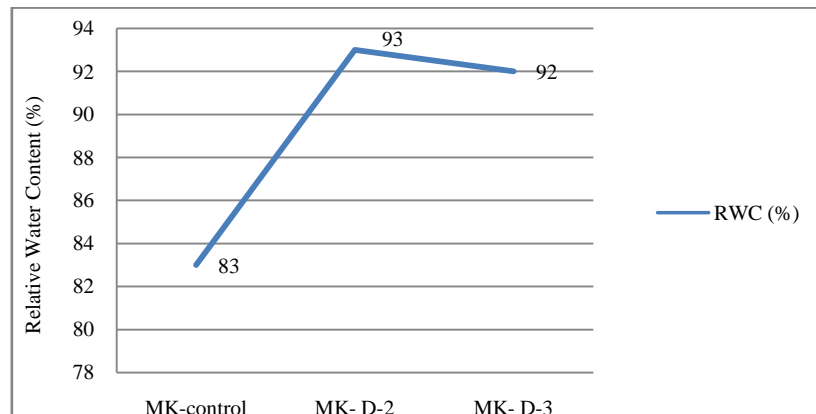


Fig. 3. Relative water content in leaf tissues of MK-control and mutant lines (MK-D-2 and MK-D-3)

It has been reported that soil moisture varies over the range of soil depths and the timing of stress. Before imposing water stress to the plants, soil moisture content was between 25% and 35% in different soil depth (10cm, 20cm and 30cm). However, soil moisture content declined about 10% to 20% after stress imposing. Matching growth duration of plants to soil moisture availability is critical to realize high seed yield [18]. Drought escape occurs when phenological development is successfully matched with periods of soil moisture availability, where the growing season is shorter and terminal drought stress predominates.

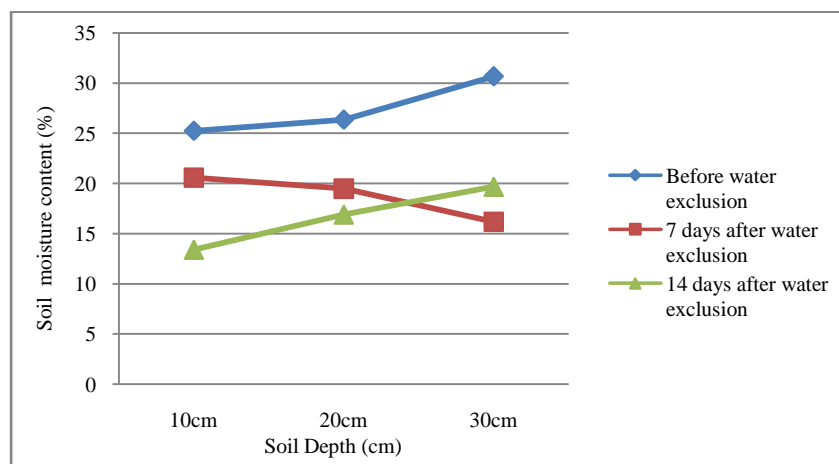


Fig. 4. Soil moisture content in different soil depths before and after water exclusion

IV. CONCLUSION

In this study, the mild drought stress in rainy season cultivation did not make severely affects in all yield component characters of both non-irradiated control plants and mutant lines. Comparison with the irradiated mutant lines, the non-irradiated control ones were not completely fertile although they can reach to flowering stage under water stress condition in dry season cultivation. Rice yield in severe stress condition is still decreasing in comparison with well-watered condition. The best two mutant lines selected were able to withhold more water in their leaves even in severe drought condition and this is one of the drought tolerant characters. Furthermore, not only future physiological screening techniques but also molecular characterization must be carried out in subsequent generation to screen stability and homozygosity. More investigations should be done on water stress at rice plant reproductive stage to better understand the physiological phenomenon that happened when this constrain occurs.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 11, November 2015

ACKNOWLEDGMENT

The author acknowledges the help from her teachers, supervisor and co-supervisor. She is also thankful to all her colleagues from Rice Research Unit, Biotechnology Research Department for their help and support. Above all, she is indebted to her parents for the great support and encouragement during this research.

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