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MUTATION BREEDING OF A RICE DWARF LINE WITH SLIGHT YIELD LOSS UNDER DROUGHT STRESS

ABSTRACT

Lack of adequate water leading to drought stress is a common constraint in upland cultivation system of rice. A rice mutant line (MT58) was developed in previous research after mutagenesis of cv. Neda by ethyl methane sulfonate (EMS) and selected for dwarfism (18 cm shorter than Neda). The extent of its molecular changes relative to parental cultivar was assessed by SSR and ISSR markers, and the response of the line along with parental cultivar and another mutant line (MTA) to mild and severe water deficit, was evaluated in a field experiment. A molecular assessment by using 41 SSR markers showed that stunt line MT58 had significant molecular difference with two other lines as confirmed by cluster analysis. ISSR assay also proved the considerable mutational effect of EMS on two mutant lines compared to original wild line. Field experiments revealed that limited irrigation caused mild to severe decrease in all the studied traits including chlorophyll contents. In both mild and severe water stress (S1 and S2) dwarf mutant MT58 had not significant differences in plant yield from parental cultivar Neda. In mild water stress cultivar Neda and mutant line MT58, respectively, showed highest (14%) and lowest (3%) yield loss, while in severe water stress mutant lines MTA and MT58, respectively, showed highest (33%) and lowest (19%) yield loss. In severe stress, cultivar Neda had highest plant height, tiller number and plant yield, and mutant MT58 had highest panicle length, total kernels per panicle, fertile kernels and chlorophyll contents. Reduction in chlorophyll content at drought stress condition was correlated with yield loss (0.64 and 0.697 for chl.a and chl.b, respectively). The results of this research obviously confirm that mutant line MT58 despite of its stunt figure did not show yield difference from its parental cultivar in drought stress.

Key words: drought, morpho-physiological, mutant, rice, yield loss

INTRODUCTION

Abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth, and development (Chutia and Borah, 2012). Rice genotypes are known to vary widely in their responses to abiot-

ic stresses, partly due to the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Zhu, 2002). Drought is an abiotic stress affecting plants at various levels of their life period. Drought not only affects plant-water relations through the reduction of water content, turgor and total water, but it also affects stomatal closure, limits gas exchange, reduces transpiration and arrests photosynthesis rates (Razak *et al.*, 2013). Negative effects of water deficit on mineral nutrition (uptake and transport of nutrients) and metabolism leads to a decrease in the leaf area and alteration in assimilate partitioning among the organs (Zain *et al.*, 2014).

Lack of adequate water leading to drought stress is a common constraint in upland cultivation system of rice. More than half of the 40 million hectares of rain-fed lowland rice worldwide suffer water deficit at some growth stages (Cabangon *et al.*, 2002). Drought stress reduces the rice growth, and severely affects the seedling biomass, photosynthesis, stomatal conductance, plant water relations and starch metabolism (Sarkarung *et al.*, 1997). Depending on timing, duration and severity of the plant water deficit, the grain yield of some rice genotypes could be reduced by up to 81% under drought (Pantuwan *et al.*, 2000). Cha-um *et al.* (2010) reported differential responses of rice genotypes to water deficit. They observed when exposed to water-deficit, panicle length and fertile grains in two tolerant varieties were stabilized, leading to greater productivity than in two sensitive cultivars.

It was reported that the approach for measuring photosynthetic traits such as chlorophyll content and chlorophyll fluorescence parameters might estimate influence of the environmental stress on growth and yield, since these traits were closely correlated with the rate of carbon exchange (Araus *et al.*, 1998; Guo and Li, 2000; Fracheboud *et al.*, 2004). Non-stomatal restrictions on CO₂ assimilation under drought stress can be effectively assessed through measuring chlorophyll based parameters (Chuti and Borah, 2012). Energetic status of the chloroplast increases as a consequence of the drought stress which has a direct relationship to that of increased amount of total chlorophyll and Chla and Chlb (Ranjbarfordoei *et al.*, 2000) among the stressed induced varieties. In response to water stress, a decrease in net CO₂ assimilation is generally observed. This effect can result from different events, such as an inhibition of electron transport activity limiting the generation of reducing power or a limitation in the metabolic activity (Guo and Al-Khatib, 2003). Rice has been identified as water deficit susceptible crop, showing negative effects especially in the reproductive stage. In rain fed paddy fields, water deficit has been reported as being a serious issue, especially in the booting stage, during which plants are particularly susceptible, leading to low crop productivity (Pantuwan *et al.*, 2002).

Since water availability will be a major constraint for paddy rice productivity in the near future, we studied the response of a dwarf mutant line of rice (recently developed in our research group using mutagenesis by ethyl methane sulfonate, EMS) along with its parental cultivar to water deficit aiming to evaluate effect of stunt figure on morpho-physiological traits, particularly plant yield in a field experiment.

MATERIAL AND METHODS

Plant material and molecular assessments

In this research an elite rice cultivar (Neda) along with two promising mutant lines (MT58 with stunt figure and MTA) developed from EMS-induced mutation of the Neda cultivar were evaluated at molecular level by using 41 SSR primer pairs (www.gramene.org; RM522, RM7300, RM6128, RM272, RM134, RM3510, RM5373, RM311, RM1146, RM3233, RM1, RM7180, RM7241, RM207, RM131, RM25, RM215, RM158, RM417, RM320, RM516, RM502, RM332, RM317, RM339, RM457, RM687, RM50, RM206, RM242, RM527, RM566, RM3873, RM283, RM505, RM527, RM106, RM171, RM412, RM1108, RM7241) and 10 ISSR primers (Table 1). Polymerase chain reaction (PCR) mixture included 5 µL of deionized water, 6 µL PCR Master Mix (SinaClone Co.), 0.25 µL of each primer (10 pg) and 0.75 µL of DNA template (15 ng). Each reaction was prepared in a 0.2-mL tubes. In the case of SSR assays, the PCR amplification started at 93°C for 5 min and continued for 35 cycles (each cycle included denaturation at 93°C for 35 s, annealing at 55°C for 30 s, extension at 72°C for 1 min). Completing the synthesis was done at 72°C for 5 min. The PCR products were separated by electrophoresis in 3.5% agarose gel and 1x TBE buffer containing 0.5 µg ml⁻¹ ethidium bromide. In the case of ISSR assays, the PCR amplification started at 93°C for 5 min and continued for 35 cycles (each cycle included denaturation at 93°C for 35 s, annealing at 47°C for 30 s, extension at 72°C for 2 min). Completing the synthesis was done at 72°C for 7 min. The PCR products were separated by electrophoresis in 1.5% agarose gel and 1x TBE buffer containing 0.5 µg ml⁻¹ ethidium bromide. a molecular size marker (100 bp ladder) was used to determine the fragments size. DNA fragment bands became visible after staining by ethidium bromide and their photos were taken under ultraviolet light by a Gel-Doc system (BioRad, USA).

Table 1

ISSR primers used in the study and their polymorphic products

No.	Primer symbol	Sequence (5'-3')	No. of polymorphic bands
1	ISSR1	(GA)7-RG	3
2	ISSR2	(CA)7-YC	4
3	ISSR3	(AG)8-T	5
4	ISSR4	(AG)8-YC	4
5	ISSR5	(GT)8-YC	1
6	ISSR6	(AC)8-YG	1
7	ISSR7	(TG)8-RC	1
8	ISSR8	(AT)7-RC	0
9	ISSR9	(CA)7-YG	3
10	ISSR10	(CA)8-RC	4

Morpho-physiological measurements

Field experiment was conducted in research farm of Gorgan University of Agricultural Sciences and Natural Resources in 2012 in a split block design with three replications. Three genotypes (Neda, MT58 and MTA) were sown in three blocks of irrigation (S0: full irrigation; S1: 1 day irrigation followed by 1 day no irrigation; S2: 1 day irrigation followed by 2 days no irrigation). Plants of each genotype were transplanted in plots of five rows with 25 cm between rows and 25 cm spacing between hills. a single 30-days-old seedling was transplanted per hill. At appropriate times some important traits were evaluated including morphological traits [plant height (PLH), panicle length (PL), total kernels per panicle (TK), fertile kernels (FK) per panicle, tiller number (TN), 100-kernel weight (HKW) and plant yield (PY)] and physiological characters [chlorophyll a (Chl.a), chlorophyll b (Chl.b) and total chlorophyll (Chl.a+b) contents]. Chlorophyll contents were determined by taking fresh leaf samples (0.1 g) from flag leaves three times in reproductive phase (start of panicle emergence, 1 day after panicle emergence and 2 days after panicle emergence). The samples were homogenized with 5 ml of acetone (80% v/v) using pestle and mortar and centrifuged at 5000 rpm. The absorbance was measured with a UV/visible spectrophotometer at 663.6 and 646.6 nm and chlorophyll contents [$\text{mg} \times \text{g}^{-1}_{\text{F.W.}}$] were calculated using the equations proposed by Porra (2002) given below:

$$\text{Chl } a = (12.55A_{663.6} - 2.55A_{646.6}) \times \frac{V \times W}{1000}$$

$$\text{Chl } b = (20.31A_{646.6} - 4.91A_{663.6}) \times \frac{V \times W}{1000}$$

where, $_{\text{F.W.}}$ is fresh weight, A is the absorbance at specific wave lengths; V is the final volume of chlorophyll extract in 80% acetone and W is the fresh weight of tissue extract.

Data analysis

SSR and ISSR score data was analyzed using NTSYSpc (version 2.2). Field data were analyzed using SAS (version 9.3) statistical package. Mean comparisons were performed using Duncan's multiple range test ($P < 0.05$).

RESULTS

SSR and ISSR assays

At all SSR loci wild-type line Neda and mutant line MTA showed identical banding patterns. However, mutant line MT58 showed different banding patterns in seven out of 41 SSR loci (RM1, RM3510, RM3233, RM1146, RM26, RM3873, and RM505). Cluster analysis placed the mutant line MT58 in

a separate group and two lines Neda and MTA in another group (Fig. 1 top); the grouping was validated by a high cophenetic correlation coefficient ($r=0.968$). However, based on ISSR markers, of 54 produced bands by 10 ISSR primers, 26 polymorphic bands (48.1%) was observed between three genotypes. Cluster analysis differentiated the two mutant lines from wild-type line Neda and they were placed in a separate group (Fig. 1 bottom); in this case also the grouping was validated by a high cophenetic correlation coefficient ($r=0.962$).

Fig. 1. Dendrograms of the studied genotypes using UPGMA method based on SSR (top) or ISSR (bottom) markers

Effect of water deficit on morphological traits

Analysis of variance showed that water stress significantly affected morphological traits including plant height (PLH) and plant yield (PY) (Table 2), indicating these traits were highly affected by irrigation regime. Genotype effect was significant for all morphological traits except for tiller number and plant yield. Effect of stress $\times$ genotype interaction was significant only for plant height.

Table 2

Analysis of variance for morphological traits in different irrigation regimes

Source	D.F.	PLH	PL	TK	FK	HKW	Till	PY
Rep	2	0.55	0.57	56.45	44.20	0.01	4.18	17.39
Stress	2	105.72**	1.65	780.81	734.04	0.02	12.65	291.28*
Ea	6	0.17	0.49	302.10	507.07	0.01	5.37	53.92
Genotype	2	847.12**	5.22**	3310.90**	2210.47**	0.35**	2.11	14.62
Stress×Genotype	4	5.42**	0.12	221.31	61.58	0.01	2.97	13.82
Eb	9	0.43	0.26	119.90	164.96	0.01	1.22	14.91

* and ** indicating significant differences at 5% and 1% level of probability, respectively

Mean's comparisons revealed that irrigation regimes had significant differences in all the studied traits except for 100-kernel weight (Table 3). As expected, maximum performance of all traits was observed in normal irrigation (S0). Mild water stress (S1) and severe water stress (S2), respectively had moderate and severe negative effects on all the studied traits. Particularly, severe water deficit (S2) compared to normal irrigation significantly decreased plant height (8 cm), total kernels per panicle (18 kernels), tiller number (2 tillers) and plant yield ($12 \text{ g} \times \text{plant}^{-1}$).

Mean's comparisons for different genotypes showed that the three studied genotypes showed identical performance for tiller number and plant yield. Elite cultivar Neda along with mutant line MTA had higher performance for plant height and 1000-kernel weight, while mutant line MT58 had higher performance for panicle length, total kernels per panicle and fertile kernels, and showed significant dwarfism (~18 cm) relative to two other genotypes (Table 3).

Table 3

Mean's comparisons of different traits as influenced by water stress and genotype

Level/Name	PLH	PL	TK	FK	HKW	Till	PY
Water stress							
S0	100.67 ^a	24.34 ^a	134.58 ^a	119.20 ^a	2.59 ^a	16.69 ^a	43.91 ^a
S1	96.56 ^b	23.71 ^b	125.96 ^{ab}	109.60 ^{ab}	2.54 ^a	16.82 ^a	39.45 ^b
S2	92.89 ^c	23.49 ^b	116.39 ^b	100.83 ^b	2.52 ^a	14.51 ^b	31.97 ^c
Genotype							
Neda	102.78 ^a	23.33 ^b	113.82 ^b	102.49 ^b	2.67 ^a	16.31 ^a	39.81 ^a
MTA	102.19 ^a	23.47 ^b	115.24 ^b	99.4 ^b	2.66 ^a	15.57 ^a	37.94 ^a
MT58	85.00 ^b	24.73 ^a	147.86 ^a	127.74 ^a	2.31 ^b	16.14 ^a	37.59 ^a

Values with common letters haven't significant differences at 5% level of probability

Further analysis on the stress × genotype interactions showed that in normal irrigation (S0) cultivar Neda had highest plant height, tiller number and plant yield (Fig. 2; Table 4), while mutant line MTA had highest 100-kernel weight. Stunt mutant line MT58 in normal irrigation had highest panicle length, total kernels per panicle and fertile kernels, and had shortest plant height. In severe water deficit (S2), all genotypes showed similar trend to what observed in mild water stress in most studied traits.

Fig. 2. Response of studied genotypes to water deficit. Left: plant height; Right: plant yield; S₀: normal irrigation; S₁: one day irrigation followed by one day no irrigation; S₂: one day irrigation followed by two days no irrigation. Columns with common letters have not significant differences at 5% level of probability

Assessment of yield loss due to water deficit revealed that in mild water stress (S₁) cultivar Neda and mutant line MT58 showed highest (14%) and lowest (3%) yield loss, respectively (Fig. 3), while in severe water deficit mutant lines MTA and MT58 showed highest (33%) and lowest (19%) yield loss, respectively.

Fig. 3. Yield loss due to water deficit of studied genotypes at two water deficit levels relative to normal irrigation condition. S₁: one day irrigation followed by one day no irrigation; S₂: one day irrigation followed by two days no irrigation

Mean's comparisons of different traits as influenced by stress $\times$ genotype interaction

Table 4

Water stress level		Genotypes		
		Neda	MTA	MT58
PL	S ₀	23.64 ^{cd}	24.03 ^{bc}	25.25 ^a
	S ₁	22.94 ^d	23.62 ^c	24.78 ^{ab}
	S ₂	23.03 ^{cd}	22.81 ^d	24.2 ^{ab}
TK	S ₀	119.10 ^{cd}	118.30 ^{cd}	167.30 ^a
	S ₁	111.16 ^d	130.28 ^c	149.55 ^b
	S ₂	107.02 ^d	104.06 ^d	133.63 ^{bc}
FK	S ₀	110.58 ^c	103.88 ^c	142.28 ^a
	S ₁	101.91 ^{cd}	107.56 ^{cd}	130.76 ^{ab}
	S ₂	90.67 ^d	91.78 ^d	115.21 ^{bc}
HKW	S ₀	2.69 ^{ab}	2.75 ^a	2.35 ^c
	S ₁	2.68 ^{ab}	2.64 ^{ab}	2.31 ^c
	S ₂	2.65 ^{ab}	2.58 ^b	2.26 ^c
Till	S ₀	17.76 ^a	15.78 ^{cd}	14.85 ^d
	S ₁	17.19 ^{ab}	16.1 ^{bc}	17.04 ^{ab}
	S ₂	13.81 ^c	13.55 ^c	15.28 ^{cd}

Values with common letters haven't significant differences at 5% level of probability

Effect of water deficit on chlorophyll contents

In the case of Chlorophyll contents ANOVA showed that water stress significantly affected Chl.a and Chl.b at all panicle emergence stages T1-T3 (Table 5). Genotype effect was also significant for these physiological traits, while effect of stress $\times$ genotype interaction was significant for Chl.a at all panicle emergence stages and for chl.b at emergence stage T1 (Table 5).

Analysis of variance for chlorophyll contents of flag leaf in reproductive phase in different irrigation regimes

Table 5

Parameters	D.F.	Type of chlorophyll					
		Chl.a			Chl.b		
Source		T1	T2	T3	T1	T2	T3
Rep	2	0.042	0.04	0.002	0.007	0.033	0.062
Stress	2	4.972**	2.818**	2.481**	4.473**	2.72**	2.514**
Ea	6	0.034	0.010	0.019	0.014	0.013	0.063
Genotype	2	0.544**	0.321**	0.364**	0.117**	0.072*	0.243*
Stress $\times$ Genotype	4	0.151*	0.132**	0.146*	0.046**	0.041	0.054
Eb	9	0.024	0.006	0.011	0.007	0.014	0.054

T1 to T3 indicate panicle emergence, 1 day after panicle emergence and 2 days after panicle emergence, respectively. * and ** indicating significant differences at 5% and 1% level of probability, respectively

Water deficit considerably affected chlorophyll contents. Chl.a and Chl.b contents were reduced under both mild and severe drought stress (Table 6). Dwarf mutant line MT58 had higher chl.a contents than that of two other genotypes at all pan-

icle emergence stages. The line also had higher chl.b contents at panicle emergence stages T1 and T2, but had not no difference from mutant line MTA at T3 stage. The analysis of stress by genotype interaction showed that at normal irrigation condition (S0) all genotypes didn't differ in chlorophyll contents (as representative, Fig. 4 relates to T1 stage of panicle emergence), while at mild stress condition (S1) two mutant lines accumulated more Chl.b relative to elite cultivar Neda, and mutant line MT58 had higher Chl.a than two other genotypes. At severe stress condition (S2) mutant line MT58 accumulated more chlorophylls, although its Chl.a was significantly higher than that of two other genotypes.

Table 6
Mean's comparisons of chlorophyll contents of flag leaf as influenced by water stress and genotype in reproductive phase

Water stress level/ Genotypes	Chl.a			Chl.b		
	T1	T2	T3	T1	T2	T3
Water stress						
S0	8.51 ^a	8.13 ^a	7.99 ^a	6.81 ^a	6.46 ^a	6.32 ^a
S1	8.05 ^b	7.68 ^b	7.54 ^b	6.34 ^b	5.92 ^b	5.62 ^b
S2	6.88 ^c	6.88 ^c	6.81 ^c	5.26 ^c	5.22 ^c	5.14 ^c
Genotype						
Neda	7.51 ^c	7.32 ^c	7.20 ^c	5.98 ^c	5.76 ^b	5.51 ^b
MTA	7.84 ^b	7.60 ^b	7.47 ^b	6.20 ^a	5.89 ^a	5.79 ^a
MT58	8.09 ^a	7.77 ^a	7.67 ^a	6.24 ^a	5.93 ^a	5.75 ^a

Values with common letters on each column haven't significant differences at 5% level of probability

Fig. 4. Reaction of the studied genotypes to water deficit in chlorophyll contents at T2 stage of panicle emergence

Correlation analysis showed that among morphological traits, panicle length (PL) had highest (0.862) significant correlation with plant yield, followed by fertile kernels (FK) per panicle (0.455), and tiller number (TN) (0.45), and all chlorophyll contents had a high significant positive correlation (>0.64) with plant yield (Table 7).

Table 7
Relationship between the studied traits as obtained by Pearson's correlation analysis

Traits	PLH	PL	TK	FK	HKW	TN	PY	Chl.a	Chl.b
PLH	1								
PL	-0.514**	1							
TK	-0.599**	0.870**	1						
FK	-0.501**	0.832**	0.949**	1					
HKW	0.923**	-0.519**	-0.602**	-0.473*	1				
TN	0.145	0.412*	0.345	0.421*	0.110	1			
PY	0.352	0.450*	0.345	0.455*	0.327	0.862**	1		
Chl.a	0.085	0.488**	0.450*	0.461*	-0.115	0.508**	0.640**	1	
Chl.b	0.252	0.457*	0.410*	0.411*	0.050	0.460*	0.697**	0.913**	1

* and ** indicating significant correlation at 5% and 1% level of probability, respectively

DISCUSSION

In this research we analyzed the molecular differences of an original line (Neda) and two mutant lines developed after mutagenesis of Neda line by using EMS chemical mutagen. As shown the mutation didn't affect most studied SSR loci, so only 7 out of 41 loci (17.1%) governed mutational changes in one of mutant lines (MT58). Wu *et al.* (2005) found no mutational changes between original rice line IR64 and nearly all morphological mutants by using 12 SSR markers. In contrast, Poli *et al.* (2013) reported polymorphism in 6 SSR loci between IR64 and its isogenic-mutant line Nagina22. These results indicate that EMS could induce an adequate number of mutated loci in mutant MT58. In another study on wheat, Zhang *et al.* (2015) reported that near 21% of SSR markers showed polymorphism between original line and an EMS-induced mutant.

In our research, in another hand, ISSR assay showed that 26 out of 54 loci (48.1%) governed mutational changes between three studied genotypes. Wannajindaporn *et al.* (2014) in a research on *Dendrobium* reported a 22.5% polymorphism in ISSR loci between original clone (Earsakul) and 28 of its mutant clones. In a work by Ahmadikhah *et al.* (2014) to study the EMS-induced salt tolerance in rice, 50% of ISSR markers showed polymorphism between wild cultivar and nine mutant lines.

In water stress assays, we found that water deficit as expected negatively imposed all evaluated morpho-physiological characters of rice plant, but the severity of loosed performance differed depending on stress level and genotype. In both mild and severe water stresses mutant dwarf line MT58 showed lowest (3% and 19%, respectively) yield loss. Grain yield under stress environment is

the primary trait for the improvement of drought tolerance. Drought effect on seed yield is due to the relation with duration of watering from flowering until physiological maturity (Midaoui *et al.*, 2003).

Photosynthesis is an essential process to maintain crop growth and development, and it is well known that photosynthetic systems in higher plants are most sensitive to drought stress (Falk *et al.*, 1996). Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate (Guo and Li, 1996). Dalal and Tripathy (2012) showed that chlorophyll content was reduced under PEG-induced drought stress in rice seedlings. Ganji Arjenaki *et al.* (2012) also observed a reduction in chlorophyll content due to water stress at anthesis stage in wheat. Exposure to drought stress leads to a significant effect in Chlorophyll.a and Chlorophyll.b contents (Ranjbarfordoei *et al.*, 2000). In our study also chlorophyll contents showed considerable sensitivity to water deficit, so that with a mild water stress all chlorophyll contents were reduced compared to normal water condition, and in a more severe water stress their reduction was more visible (Table 3). Genotype reaction to water deficit was well observable in their chlorophyll accumulation at drought stress condition; this issue can be particularly observed for chl.a content (Table 3). Cha-um *et al.* (2010) and Mafakheri *et al.* (2010) reported a marked decrease in all physiological parameters due to drought stress in drought-sensitive rice and chickpea genotypes, respectively. A reason for decrease in chlorophyll content as affected by water deficit is that drought stress by producing reactive oxygen species (ROS) such as O₂⁻ and H₂O₂, can lead to lipid peroxidation and consequently, chlorophyll destruction (Mirnoff, 1993; Foyer *et al.*, 1994; Hirt and Shinozaki, 2004). Also, with decreasing chlorophyll content due to the changing green color of the leaf into yellow, the reflectance of the incident radiation is increased (Schelmmmer *et al.*, 2005).

Correlation analysis showed a clear positive relationship (>0.64) between plant yield and chlorophyll contents (Table 4), probably due to maintaining stay-green state despite of water deficit, especially in two less sensitive mutant lines, MTA and MT58. Although there is an argument about whether a higher chlorophyll content (i.e., stay green trait) contributes to yield under drought conditions or not (Blum, 1998), many studies indicated that stay-green is associated with improved yield and transpiration efficiency under water-limited conditions in sorghum, maize, wheat, barley and rice (Benbella and Paulsen, 1998; Borrell *et al.*, 2000; Haussmann *et al.*, 2002; Verma *et al.*, 2004; Li *et al.*, 2006; Cha-um *et al.*, 2010).

CONCLUSION

Based on molecular studies in this research it can be concluded that EMS didn't alter most studied SSR loci, while it introduced adequate mutational alterations in ISSR regions. Field experiments revealed that water deficit negatively affects all evaluated morpho-physiological characters of rice plant and that maintaining yield under water stress mainly is possible via continuing pho-

tosynthesis activity and staying green the leaves. With regard to all aspects of findings of our research, mutant line MT58 despite of its stunt figure did not show yield difference from its parental cultivar under drought stress.

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