

Comparative Morphology, Photoperiodism, and Yield of KDML105 Rice (*Oryza sativa*) and Its Mutants

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ABSTRACT

Climate change and decreasing in farmlands threaten global rice production. The productivity and grain quality of KDML105 (*Oryza sativa* 'KDML105'), one of Thailand's famous scented rice is depending on photoperiod and cultivating conditions. Cultivar improvement using induced mutagenesis has previously been performed and screening of candidate mutant lines (MT1, MT3, MT4, MT5, MT6) were then investigated in comparison with KDML105 to gain M6 generation. Comparative morphological features revealed that five mutant lines showed semi-dwarf shoot with erected leaves, higher tiller numbers, and compact clump when compared to KDML105. Interestingly, the variations of photoperiodism of flowering were observed among mutants and its original. All five lines of candidate mutants showed flowering unaffected by day length even under long-day or short-day that required only 47-85 days after transplanting to reach the flowering date. In contrast, KDML 105 showed flowering only under short-day conditions. On the other hand, three candidate mutants (MT3, MT5 and MT6) after screening for salinity tolerance were further selected to grow for M6 seed production. The tiller numbers, spikelet numbers, total grain yield, and grain weight obtained from MT3, MT5 and MT6 mutants were significantly more than those obtained from KDML 105. The mutants obtained in this study are characterized as intermediate photoperiodism with shortened production time. It is also suggested to further investigate the grain quality to examine the overall properties of these mutants prior to use in the rice breeding program.

1. Introduction

Thai jasmine rice (Kao Dawk Mali 105, KDML105) is one of the important rice traits for Thailand's Economy. The unique scent with high cooking quality makes it popular among consumers and refers to a high marketable price in the international rice traits. The data from Thailand ministry of commerce showed that the export of KDML105 rice steadily increased from 2015 to around 1,607,356 tons in 2019 (Chuaykerd *et al.* 2020). Among Thai scent rice cultivars, KDML 105 has been identified as the cultivar that produces the highest grain quality (Vanavichit *et al.* 2018). However, it has inadequate productivity and still has limitations due to its phenotype's hump to its production such as long shoot length with droopy clump (Yan *et al.* 2012) makes it susceptible to lodging.

Lodging of rice caused a problem in rice production, it leading to poor grain filling, poor grain quality and lowering the efficiency of harvesting (Jinger and Dhar 2018). Droopy leave reduced light harvesting for photosynthesis under high-density cultivation (Wickramasinghe *et al.* 2021). Moreover, KDML105 is a photoperiod-sensitive rice cultivar which flowering is induced by short-day photoperiod. Traditionally, it is cultivated once a year, started during the monsoon in July (Khotasena *et al.* 2022) and harvested in November (Vanavichit *et al.* 2018).

The current situation of decreasing rice farmland due to land conversion, expansion of urban areas (Chai *et al.* 2019; Widyawati *et al.* 2021) and climate changes affected global rice production. Most KDML105 production is located in the northeast of Thailand where some areas are prone to high salinization (Vanavichit *et al.* 2018). It predicted that climate change will accelerate soil salinization

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(Eswar *et al.* 2021) and might threaten KDML105 production. KDML105 is considered a moderately salt-susceptible cultivar in which productivity is reduced by exposure to moderate salt stress ($EC = 4.0 \text{ dSm}^{-1}$) (Sangwongchai and Thitisaksakul 2019). Salt stress leads to osmotic stress that induces stomata to close which restricted the CO_2 available for photosynthesis (Hussain *et al.* 2017). Furthermore, long-term exposure to NaCl reduce chlorophyll concentration in the leaves which limits the ability of the plant to harvest light energy for photosynthesis (Zahra *et al.* 2022). Restriction of sugar supply from the leave due to reduction in photosynthesis affected by salt stress at reproductive stage causes sterility and reduces grain weight production in rice (Abdullah *et al.* 2001).

To avoid the demand gap in the future and sustain food security, it is indispensable to increase KDML105 production and improve its tolerance to salt stress, as well. The global impact of mutation-derived varieties of crop plants with consumers' preferences has been used to improve rice properties. Many existing KDML105 mutants with property improvements have been reported such as Ser (Yan *et al.* 2012), PKOS, BKOS (Boonrueng *et al.* 2013), RD 6 and RD15 (Mackill *et al.* 1996). Nevertheless, these mutants were evaluated according to their potential to improve KDML105 production. In this research, candidate mutants of KDML105 were evaluated according to its responses to salinity stress and photoperiodism.

2. Materials and Methods

2.1. Plant Materials

Seeds of KDML105 rice (*Oryza sativa* ssp. Indica 'KDML105') were obtained from Pathum Thani Rice Research Center, Thailand (KDML 105; GS. No. 27748). The mutant lines used in this research (lines; MT1, MT3, MT4, MT5 and MT6) were M4 generation of KDML105 mutants obtained from combination treatments of ethyl methyl sulphonate (EMS) and gamma irradiation (Theerawitaya *et al.* 2011). Each tested line was germinated from selected seed and then aseptically sub-cultured for *in vitro* multiplication. The plantlets with complete shoot/root formation were transplanted to the soil-containing pots (one plant per pot) and incubated

in the greenhouse before being used for the experiments.

2.2. Cultivation Conditions

After transplantation, the rice pots of KDML105 and mutants were subsequently cultivated in the greenhouse with good fertigation at the location site of 3.7647355, 100.523256. The morphological characterizations of the transplants were determined at both vegetative and reproductive stages. The number of tillers and the clump appearances were observed for 60 days after transplantation. Shoot length and flag leaf developments were observed at the grain-filling stage. To evaluate the photoperiodism responses, treatments of transplantation at three different periods of greenhouse-grown pot plants were conducted. The transplantation date of crop 1, crop 2 and crop 3 were performed in the same year but started at different ranges of middle January, May or August, respectively. The record of the photoperiod of culturing location is referred to <https://www.timeanddate.com/sun/thailand/bangkok>. The date of flowering was recorded when fifty percent of the panicles were partially exerted from the leaves sheet of the flag leaves (Janwan *et al.* 2013). All plants were harvested about 35 days after heading, and the percentage of fertility and grain weight were recorded.

2.3. Evaluation of Rice Mutants in Susceptibility to NaCl Stress at Early Reproductive Stage

The tested rice lines were transferred from *in vitro* culture to the soil in a plastic pot until the end of the booting stage in the greenhouse and then treated with or without 150 mM NaCl for 15 days. The solution was replaced every two days to maintain salt concentration. The photosynthesis was recorded at the end of the treatment using a portable photosynthesis system (Model LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). The flag leaves were sampled for pigment analysis. One hundred grams of leaves were grounded into powder followed by pigment extraction using 95.5% acetone at 4°C for 42 h. The absorbance of the solution was measured using a UV-vis spectrophotometer at wave length 644 and 662 nm. The concentration of the chlorophyll was measured using the following formula (Lichtenthaler 1987):

$$\begin{aligned}
 [\text{Chla}] &= 9.784 \text{ D622} - 0.99 \text{ D644} & (1) \\
 [\text{Chlb}] &= 21.42 \text{ D644} - 4.65 \text{ D622} & (2) \\
 \text{Total Chl} &= [\text{Chla} + \text{Chlb}] & (3)
 \end{aligned}$$

After salt treatment, rice was irrigated with tap water until the harvesting period.

2.4. Statistical Analysis

The experimental design used for mutants' characterization was a complete randomized design with three replications while the effect of salt stress at the early reproductive stage was evaluated using a 6×2 factorial in a complete randomized design. The different mean values of the treatment were analyzed by Duncan's Multiple Range Test (DMRT) and analyzed by SPSS software (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Evaluation of Morphological Growth and Development Among KDML105 and Its Mutants

The comparisons of shoot length, tiller numbers, characteristics of rice clump and panicle among KDML105 and five candidate mutants were demonstrated (Figure 1). KDML105 rice has a shoot length > 140 cm, while five mutant lines showed a semi-dwarf shoot length of about < 120 cm (Figure 1A). All candidate mutants produced more tillers than KDML 105 (Figure 1B), especially MT1 and MT6 had the highest tiller productions. The average tiller numbers produced by greenhouse-grown transplants of KDML 105 was 6 ± 0.58 tillers per

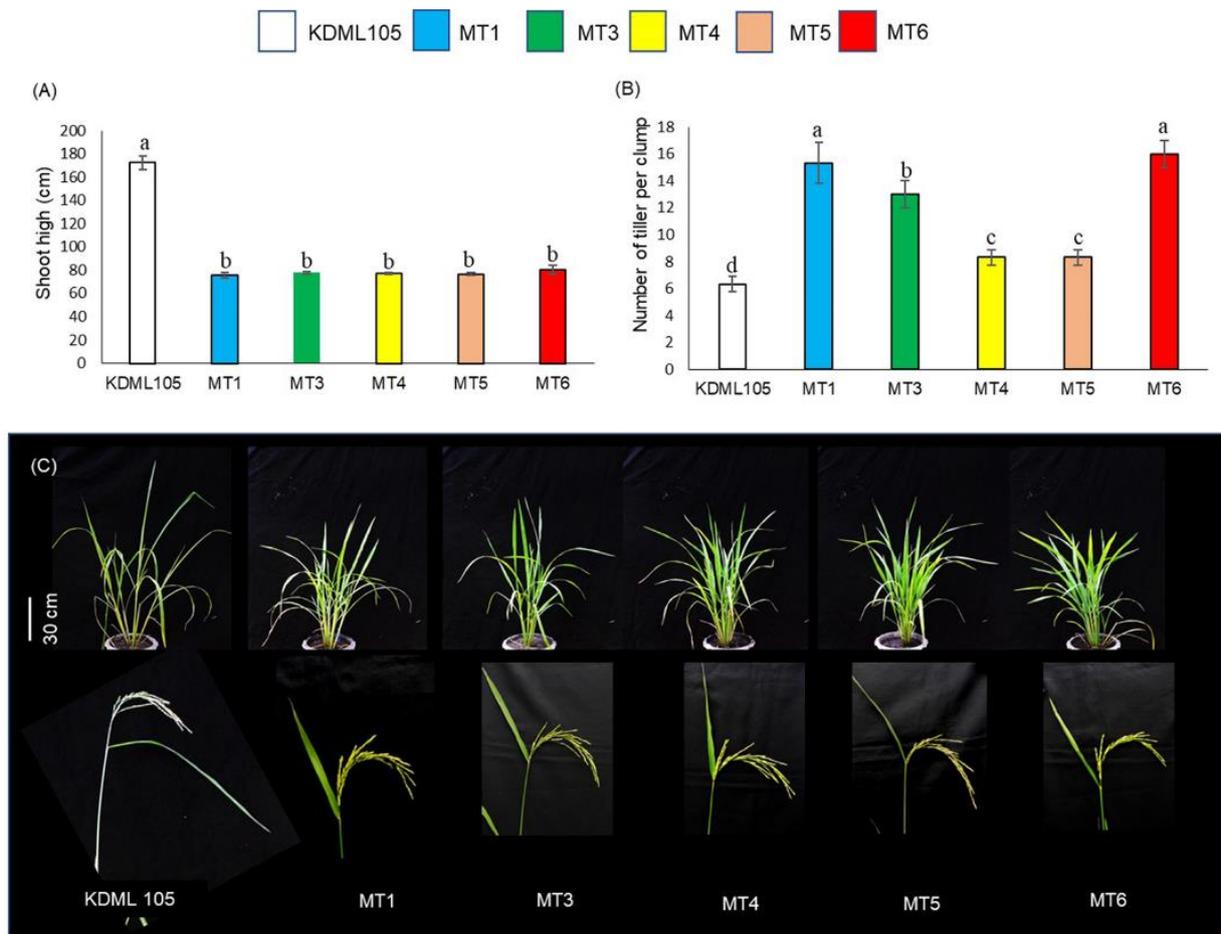


Figure 1. The morphological comparisons of KDML105 and five mutant lines (MT1, MT3, MT4, MT5, MT6). The shoot length (A) was measured at the grain filling stage; and numbers of tiller/clump (B) were recorded after 60 days of transplantation. The clump appearances, flag leaf and panicle development were compared (C). Different letters present at top of the bar graphs in Figure 1 (A) and (B) indicated the highly significant differences of mean (\pm SD) analyzed by DMRT at $p \leq 0.01$ using three biological replications (1 pot/1 replication) with three experimental replications

clump, while MT1, MT3, MT4, MT5 and MT6 produce approximately 2–9 folds of tiller numbers per clump more than their original KDML105. Moreover, all mutants have compact clumps with erected leaf (Figure 1C). In opposite, KDML 105 has a spreading shoot, loosely clump and droopy leaf.

3.2. Comparison of Flowering Date and Yield between KDML105 and Its Mutants

Time to the flowering of KDML105 and its mutants were affected by the date of transplanting (Figure 2). In this research, crop 1 of KDML cultivated in middle of January (Figure 2B) still in the vegetative stage

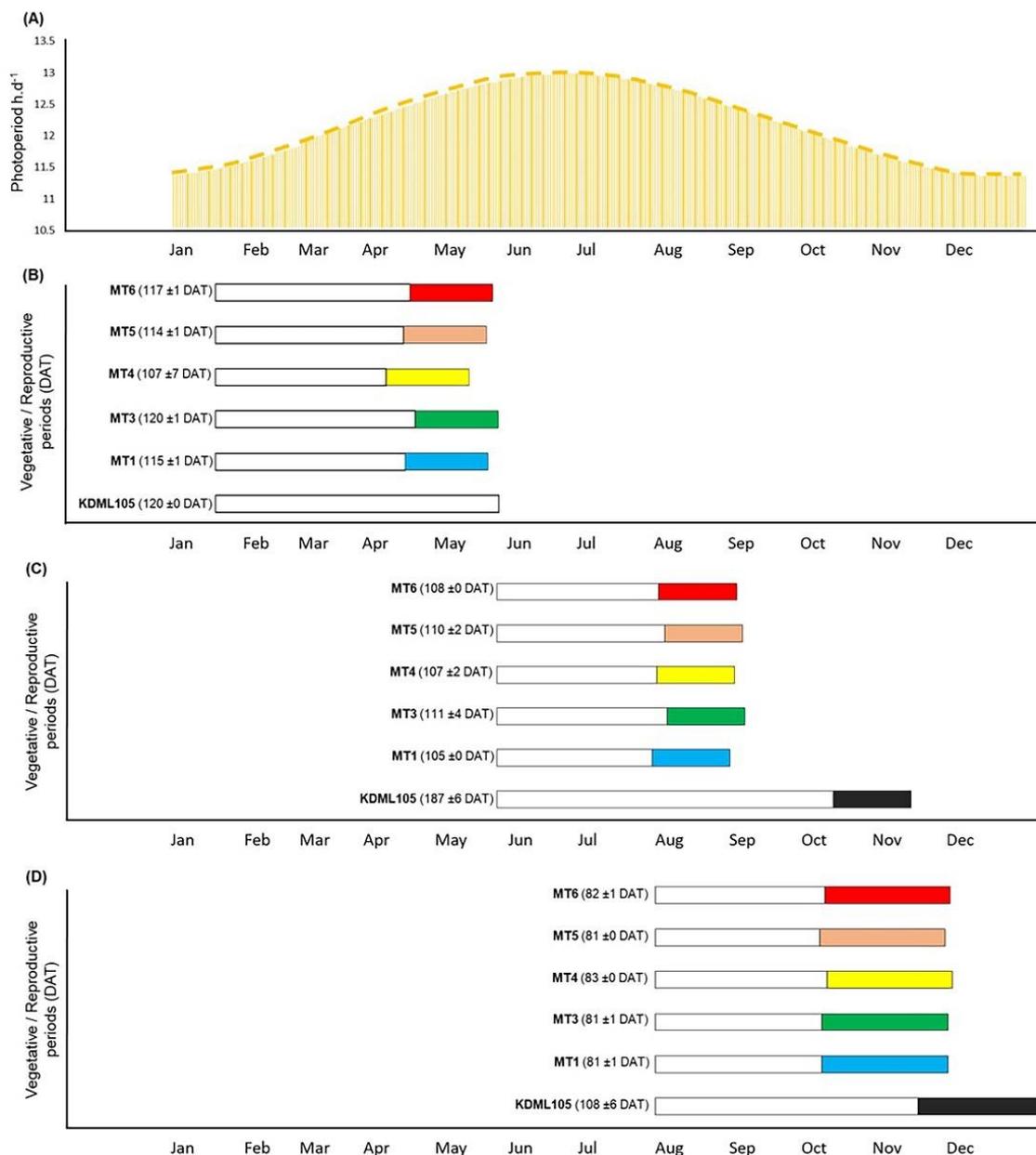


Figure 2. Photoperiod affected the panicle development of KDML105 and five mutant lines (MT1, MT3, MT4, MT5 and MT6) according to different cultivation periods. The *in vitro* plants were transferred to the soil pots and grown in the greenhouse at different periods. The vegetative and reproductive periods were recorded according to three different transplanting time in the same year as; crop 1 (B), crop 2 (C) and crop 3 (D). The record of photoperiod (Timeanddate 2022) was referred according to the location site at 3.7647355,100.523256. The white bar represented the period of vegetative stage, whereas the filled bar represented the period of reproductive stage. The start of the reproductive stage was determined when 50% of panicle (head) emerge from flag leaf. The numbers in the parentheses after code of tested rice indicated the means (\pm SE) of harvesting times (DAT = days after transplanting) > the experiments were conducted using three biological replications (1 pot/1 replication)

until the experiment was ended in May. For crop 2 and crop 3 of KDML105 that were cultivated in May (Figure 2C) and August (Figure 2D), the flowering occurred in October and November, respectively when the photoperiod was less than 12 h.d⁻¹ (Figure 2A). The flowering time of KDML105 transplanting in May (152±6 DAT) was 2 times more than the flowering date of KDML105 transplanted in August (73±6 DAT). In our experiments, all candidate mutants showed successive flowering with grains in all tested crop periods (Figure 2B-D). All five mutant lines of KDML105 that were cultivated in January or May flowered during long-day photoperiod conditions, while mutants cultivated in August flowered during short-day photoperiod. The mutants that were transplanted in August have a shorter time to flowering (~ 46-48 days) when compared to those

transplanted in January and May which need about 70 to 85 days for flowering.

Rice production is determined by the ability to produce filled grains, which depends on its fertility. In our experiment using the greenhouse-grown rice pots, the percentages of fertility and yields increased from crop 1 to crop 2 and crop 2 to crop 3 (Figure 3). KDML105 grown in crop 1 failed to produce panicle when the experiment was terminated on late May. As result, there has no fertility (%) was detected in KDML105 at crop 1. It was noted that five lines of mutant could generate fertility in crop 1, even at a low percentage of less than 20% (Figure 3A). In regards to the results from Figure 2B and C, the mutants of crop 1 and crop 2 could flowering but had slightly different flowering-date under long-day photoperiod.

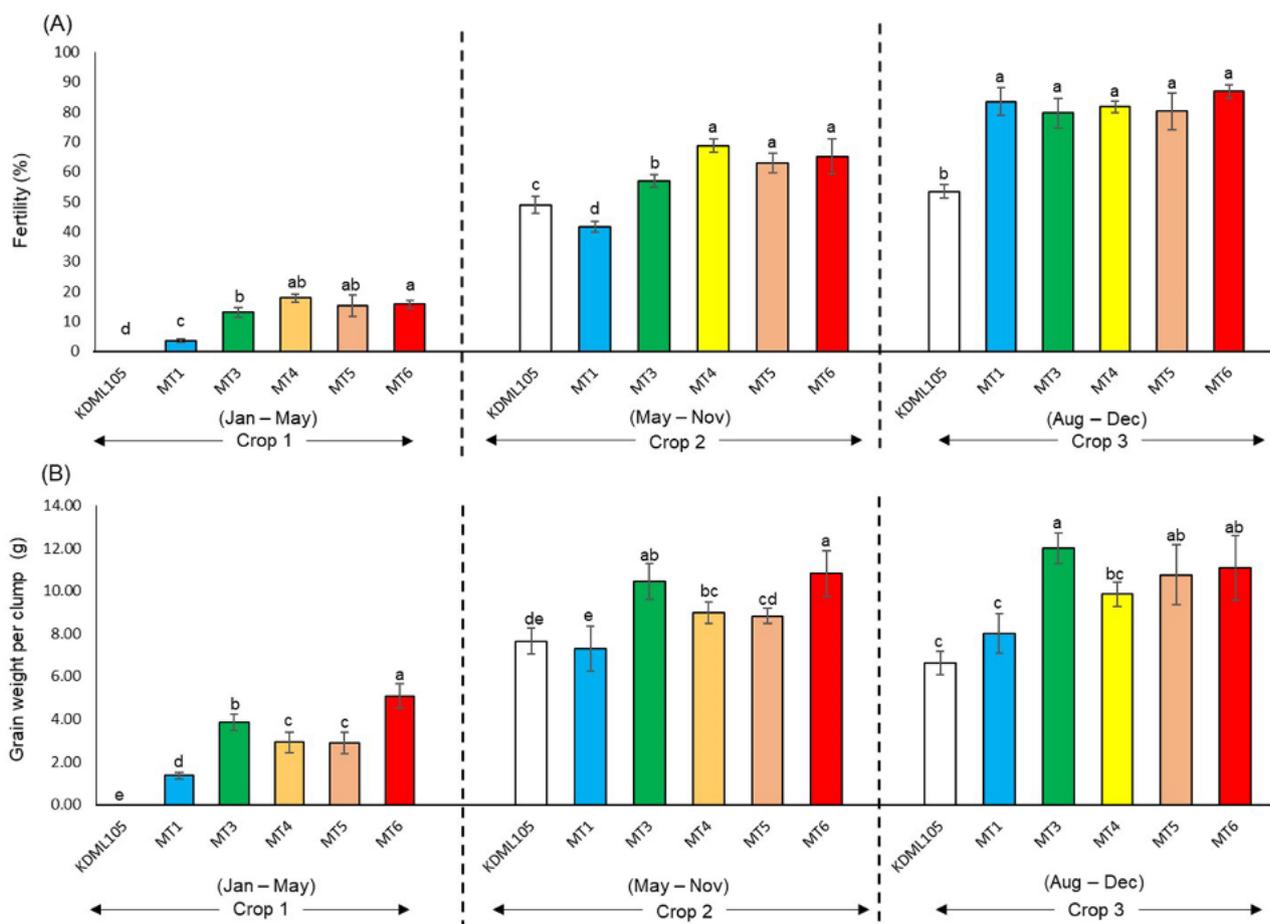


Figure 3. Percentages of fertility (A) and grain weight per clump (B) of KDML105 and five mutant lines (MT1, MT3, MT4, MT5 and MT6) recorded at different cultivation periods. The *in vitro* plants were transferred to the soil pots and grown in the greenhouse at different periods; as crop 1 (January-May; 120 days), crop 2 (May-November; 187 days) and crop 3 (August-December; 108 days) of same year. Different letters indicated highly significant differences within crop-period of mean (±SD) analyzed by DMRT at p<0.01 using three biological replications (1 pot/1 replication) with three experimental replications

3.3. Effect of NaCl Stress on Physiological Responses, Fertility Percentage and Grain Weight

All physiological parameters of rice evaluated in this study, including stomatal conductance (gs), total chlorophyll content (total chl) in the leaf and net photosynthetic rate (Pn), were affected by 150 mM NaCl stress at the early reproductive stage (Table 1). Among tested lines, MT1 showed the highest gs reduction (73.3%) followed by KDML105 (70.3%), MT4 (66.0%), MT6 (64.7%), MT3 (53.3%) and MT5 (50.6%). All mutants showed lower chlorophyll reduction (<50%) than KDML105 (66%), among the mutants MT3 showed the highest chlorophyll reduction (41%). Evaluation of the net photosynthetic rate (Pn) of KDML105 and its mutants showed a significant reduction of Pn after being treated with 150 mM NaCl. Although under the condition without salt stress (0 mM NaCl), the highest Pn was observed in line MT1, the MT3 line showed its high efficiency to retain Pn after being treated with 150 mM NaCl stress, followed by MT6, KDML105, MT5, MT4 and MT1, respectively.

At the early reproductive stage, salinity stress significantly affected the fertility and yield of KDML105 and its mutants (Table 1). The fertility of KDML 105, MT1, MT3, MT4, MT5 and MT6 treated with 150 NaCl were 73.4%, 47.4%, 39.5%, 59.5%, 5.9% and 31.5% lower than those cultivated without NaCl (0 mM). In this research, MT6 showed higher efficiency to sustain the yield under salt stress, the

reduction percentage of grain weight was the lowest (41%) followed by MT3 (45%), MT5 (51%), MT1 (63%), MT4 (67%), whereas KDML105 showed the highest yield reduction (74%) as shown in Table 1.

4. Discussion

4.1. Evaluation of Morphological Growth and Development Among KDML105 and Its Mutants

Shoot architecture has a direct impact on rice productivity. Rice with the tall shoot loosely clump, such as KDML105, is susceptible to lodging at the reproductive stage, leading to grain loss (Yan *et al.* 2012). In the present study, all KDML105 mutants have semi-dwarf shoots (Figure 1). According to a previous report, rice with semi-dwarf shoots is more resistant to lodging at the reproductive stage (Yan *et al.* 2012; Okuno *et al.* 2014). Moreover, mutants have compact clumps with more tiller per clump than KDML105. At the reproductive stage, the panicle develops from the apical meristem of the rice tiller. For that reason, the tiller number is associated with rice productivity. The compact tiller-clump type is an agronomic character that gives high-density planting advantages (Li *et al.* 2019), which tolerate to lodging and benefit for mechanical harvesting. In addition, erected leave increases the light intercept of the leaf canopy leading to better photosynthesis in high-density cultivation (Hussain *et al.* 2017).

Table 1. The effect of NaCl stress (150 mM) during early reproductive stage on stomatal conductance (gs), total chlorophyll (chl) contents and net photosynthesis rate (Pn), fertility (%) and grain weight of KDML105 and five mutant lines (MT1, MT3, MT4, MT5, MT6). The gs, chl and Pn were determined after 15 days of salt treatment

NaCl stress	Rice line	gs ($\mu\text{mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Total Chl ($\mu\text{g}\cdot\text{g}^{-1}\text{FW}$)	Pn ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{S}^{-1}$)	Fertility (%)	Grain weight (g)
0 mM	KDML 105	0.71±0.11 ^{ab}	630.9±25.0 ^a	11.6±1.27 ^c	40.9±4.30 ^d	3.77±0.19 ^c
	MT1	0.85±0.07 ^a	357.8±29.4 ^d	15.5±2.01 ^a	68.2±6.00 ^{ab}	5.43±0.13 ^b
	MT3	0.77±0.09 ^{ab}	479.4±24.4 ^b	11.2±1.49 ^{cd}	68.6±5.60 ^{ab}	5.21±0.18 ^b
	MT4	0.81±0.08 ^a	426.6±32.4 ^c	13.1±0.62 ^{bc}	67.5±6.90 ^{ab}	5.61±0.40 ^b
	MT5	0.62±0.16 ^b	371.0±49.9 ^d	14.4±1.52 ^{ab}	69.8±11.2 ^{ab}	7.10±0.27 ^a
	MT6	0.72±0.16 ^{ab}	323.1±12.1 ^{de}	11.2±1.63 ^c	79.4±2.50 ^a	5.61±0.17 ^b
150 mM	KDML 105	0.21±0.06 ^c	214.7±8.30 ⁰	6.7±0.52 ^f	10.8±0.60 ^f	0.98±0.14 ^f
	MT1	0.23±0.04 ^c	279.5±31.3 ^e	6.6±0.31 ^f	35.9±10.9 ^{de}	2.03±0.32 ^e
	MT3	0.36±0.05 ^c	281.7±27.8 ^e	9.2±0.54 ^{de}	41.5±3.40 ^d	2.85±0.37 ^d
	MT4	0.28±0.08 ^c	298.2±24.8 ^e	7.2±0.27 ^{ef}	27.3±9.80 ^e	1.85±0.42 ^e
	MT5	0.30±0.05 ^c	298.2±24.8 ^e	9.2±1.53 ^{de}	65.6±11.5 ^{bc}	3.51±0.28 ^c
	MT6	0.25±0.05 ^c	283.8±21.4 ^e	6.9±1.44 ^f	54.4±1.50 ^c	3.32±0.10 ^c
Significant level						
	Rice line	ns	**	**	**	**
	NaCl stress	**	**	**	**	**
	Line x Stress	ns	**	**	**	**

4.2. Comparison of Flowering Date and Yield between KDML105 and Its Mutants

Harvesting date not only refers to annual rice production but is also used for cost production. The harvesting date of rice is highly affected by the time of flowering. In general, the harvesting date was 35 days after the flowering date (Humayun *et al.* 2013). KDML105 is short-day rice that flowering induced by short-day photoperiod (<12 h photoperiod). The cultivation of rainfed rice as KDML105, it traditionally starts to grow in June, flowering in October when the photoperiod is less than 12 h.d⁻¹, and harvesting in November (Vanavichit *et al.* 2018). Moreover, Biswas and Ntanos (2002) reported that KDML105 transplanted in July took a longer duration to reach the flowering date than those transplanted at the end of August. In agreement with the previous report, KDML105 in this study flowering during short-day photoperiod with KDML105 transplanted in May took longer time to flowering than transplanted in August (Figure 3).

In contrast to KDML105, all mutants have intermediate responses to photoperiodism (Figure 3), which means being able to flower under long-day and short-day photoperiods, even though the flowering date is still influenced by photoperiod (Mackill *et al.* 1996). Additionally, our candidate mutants (MT1, MT3, MT4, MT5 and MT6) have a shorter harvesting time than KDML105 in all cultivation periods (Figure 3), which is one of the remarkable characteristics. The mutants transplanted in May or August could be harvested at 72-82 and 25-27 days earlier than KDML105 transplanted in the same period. A combination trait with short periods of cultivation and intermediate response to photoperiod is useful for increasing annual rice production by multiple cropping in a single year and reducing the costs of rice production.

Spikelet fertility influences rice grain weight by determining the number of filled grains. Spikelet fertility can be affected by temperature and photoperiodism (Zhu *et al.* 2020). All mutants in crop 1 and crop 2 flowering during long day photoperiod, but their fertility in crop 1 was more than 30% lower than crop 2. This result suggested that the low fertility (%) of mutants in crop 1 in Figure 3A might not cause by photoperiod or duration of cultivation. The previous reports revealed that spikelet fertility was associated with ambient temperature during heading (Jagadish *et al.* 2007; Song *et al.* 2022).

Fertility percentages of IR64 decreased when the temperature during anthesis was increased from 29.6 to 33.7°C. At a temperature above 29.6°C, the fertility decreased by about 7% per one degree Celsius of an increase in ambient temperature (Jagadish *et al.* 2007). Therefore, the mutants that cultivated in crop 1, crop 2 and crop 3 were flowering in April, July and October, respectively, where the ambient temperatures (min-max) in Bangkok during that period were ranged from 27-36°C, 26-34°C and 25-33°C, respectively (Timeanddate 2022). This might be indicated that the low fertility and yield of mutants obtained from crop 1 were highly affected by temperature stress during the hot season of April.

Rice needs optimum vegetative duration to produce maximum yield was reported by (Vergara *et al.* 1966). Shortening the vegetative stage under their optimum duration can lead to yield penalty (Vergara *et al.* 1966; Promchote *et al.* 2022). KDML105 cultivated in May (crop 2) has a longer harvesting date (Figure 2C) with higher yield (Figure 3B) than KDML105 cultivated in August (crop 3) even though they have a similar percentage of fertility (Figure 3A). This result might suggest that the lower productivity of KDML105 in crop 3 compared to crop 2 might cause by the reduction in the vegetative period. On the opposite, the shorter duration of the vegetative stage between crop 2 and crop 3 (Figure 2A and B) didn't have a negative impact on the grain yields of mutants (Figure 3B). This result demonstrated that the vegetative duration in crop 3 was enough to support grain development during the reproductive stage of mutants.

4.3. Effect of NaCl Stress on Physiological Responses, Fertility Percentage and Grain Weight

At the beginning of salt stress, plants experience a water deficit which induces the production of abscisic acid (ABA). In the leaf, ABA prevents water loss by stimulating stomata to close leading to reduce stomatal conductance (Bharath *et al.* 2021). In the long-term, salt stress trigger of chlorophyll degradation in the leaves. Generally, low stomatal conductance reduces CO₂ supply for photosynthesis while low chlorophyll concentration in the leaf reduces the ability of the plant to harvest energy for photosynthesis (Zahra *et al.* 2022). In this research, the reduction of both stomatal conductance and chlorophyll content were observed in all rice

lines treated with 150 mM NaCl, which lead to the reduction in net photosynthesis rate (Table 1). The comparison among three parameters (g_s , total chl and Pn), the reduction of Pn under salt stress is more consistent with the reduction of g_s than total chl. This phenomenon was clearly observed in KDML105, MT1 and MT6. This result might suggest that the restriction of photosynthesis of those lines under salt stress is predominantly affected by the limitation of CO₂ supply through stomata. Among the rice lines, MT3 showed its high efficiency to retain Pn after being treated with 150 mM NaCl stress, even though it has a moderate ability to maintain g_s and total chl. This result might suggest that there are factors other than g_s and total chl that also contribute to the ability of MT3 to maintain the high efficiency of Pn under 150 NaCl stress.

The reduction of rice fertility, when exposed to salt stress at the reproductive stage, was also reported by Abdullah *et al.* (2001), Boriboonkaset *et al.* (2013) and Gerona *et al.* (2019). The previous report showed that salt stress at the reproductive stage reduced pollen viability and led to spikelet sterility of salt-stress-rice (Abdullah *et al.* 2001; Gerona *et al.* 2019). Moreover, limiting of the carbohydrate supply necessary for the development of rice grain has also caused rice sterility (Abdullah *et al.* 2001). Reduction in photosynthesis of KDML105 and its mutants during salt stress further limits the carbon supply and salt stress generates low water-used efficiency of both intra- and intercellular levels due to unbalancing osmotic pressure. Thereby, plant sterility was generated under NaCl stress and reflected to reducing the percentage of fertility. Among tested lines, MT5 showed the highest efficiency to retain its fertility (%) under NaCl stress, the reduction of fertility was reduced only 4.2% which is lower than MT6 (25%), MT3 (27.1%), KDML105 (30.1%), MT1 (32.3%) and MT4 (40.2%).

The previous report showed a reduction in rice fertility under salt stress followed by a yield loss in rice (Boriboonkaset *et al.* 2013; Gerona *et al.* 2019). Similar phenomena were also found in this research at five rice lines including KDML105, MT1, MT3, MT4 and MT6 (Table 1). An interesting finding was observed in MT5, even though its fertility didn't significantly affect by salt stress, its yield was reduced by about 51% under salt stress. This condition might cause by the reduction of carbohydrate supply from photosynthesis to developing grain under salt

stress or competition for carbohydrates between developing grain and stress defence mechanism (Boriboonkaset *et al.* 2013), which might reduce the starch accumulation in developing grain and finally reduce the grain weight per plant.

Considering all parameters above, three mutants, MT3, MT5 and MT6 showed interesting performances under 150 mM NaCl than KDML 105 and the other two mutants. They could generate more grain weight under salt stress either by maintaining chlorophyll content, photosynthesis, plant fertility or a combination of them.

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References

- Abdullah, Z., Khan, M.A., Flowers, T.J., Khan, M.A., 2001. Causes of sterility in seed set of rice under salinity stress. *J. Agronomy and Crop Science*. 187, 25-32.
- Bharath, P., Gahir, S., Raghavendra, A.S., 2021. Abscisic acid-induced stomatal closure: an important component of plant defense against abiotic and biotic stress. *Frontiers in Plant Science*. 12, 1-18. <https://doi.org/10.3389/fpls.2021.615114>
- Biswas, P.K., Ntanos, D., 2002. Geographical diversity and its influence on rice yield. *Kasetsart J. (Nat. Sci.)*. 36, 345-352.
- Boonrueng, N., Anuntalabhochai, S., Jampeetong, A., 2013. Morphological and anatomical assessment of KDML 105 (*Oryza sativa* L. spp. indica) and its mutants induced by low-energy ion beam. *Rice Science*. 20, 213-219. [https://doi.org/10.1016/S1672-6308\(13\)60129-8](https://doi.org/10.1016/S1672-6308(13)60129-8)
- Boriboonkaset, T., Theerawitaya, C., Yamada, N., Pichakum, A., Supaibulwatana, K., Cha-um, S., Takabe, T., Kirdmanee, C., 2013. Regulation of some carbohydrate metabolism-related genes, starch and soluble sugar contents, photosynthetic activities and yield attributes of two contrasting rice genotypes subjected to salt stress. *Protoplasma*. 250, 1157-1167. <https://doi.org/10.1007/s00709-013-0496-9>
- Chai, J., Wang, Z., Yang, J., Zhang, L., 2019. Analysis for spatial-temporal changes of grain production and farmland resource: Evidence from Hubei Province, central China. *Journal of Cleaner Production*. 207, 474-482.
- Chuaykerd, T., Yao, S., Khamphilavong, S., Tuyen, H.T., 2020. The impact factors of thai jasmine rice export to international market. *Open Journal of Business and Management*. 8, 1113-1121. <https://doi.org/10.4236/ojbm.2020.83071>
- Eswar, D., Karuppusamy, R., Chellamuthu, S., 2021. Drivers of soil salinity and their correlation with climate change. *Current Opinion in Environmental Sustainability*. 50, 310-318. <https://doi.org/10.1016/j.cosust.2020.10.015>

- Gerona, M.E.B., Deocampo, M.P., Egdane, J.A., Ismail, A.M., Dionisio-Sese, M.L., 2019. Physiological responses of contrasting rice genotypes to salt stress at reproductive stage. *Rice Science*. 26, 207–219. <https://doi.org/10.1016/j.rsci.2019.05.001>
- Humayun, M., Baktiar, K., Siddique, M.A., Khalequzzaman, M., Bhuiya, A., Baktiar, M., Siddique, M.A., Khalequzzaman, M., Bhuiya, A., Islam, M.Z., 2013. Effect of maturity period and harvesting time on quality and yield in breeder seed of rice (*Oryza sativa* L.). *Eco-friendly Agril.* 6, 249–252. <https://www.researchgate.net/publication/276156257>
- Hussain, S., Zhang, J.hua., Zhong, C., Zhu, L.Feng., Cao, X., Chuang., Yu, S.Miao., Bohr, J.A., Hu, J.Jie., Jin, Q.Yu., 2017. Effects of salt stress on rice growth, development characteristics, and the regulating ways: a review. *Journal of Integrative Agriculture*. 16, 2357–2374. [https://doi.org/10.1016/S2095-3119\(16\)61608-8](https://doi.org/10.1016/S2095-3119(16)61608-8)
- Jagadish, S.V.K., Craufurd, P.Q., Wheeler, T.R., 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany*, 58, 1627–1635. <https://doi.org/10.1093/jxb/erm003>
- Janwan, M., Sreewongchai, T., Sripichitt, P., 2013. Rice breeding for high yield by advanced single seed descent method of selection. *Journal of Plant Sciences*, 8, 24–30. <https://doi.org/10.3923/jps.2013.24.30>
- Jinger, D., Dhar, S., 2018. Crop lodging: its causes and management for sustainable production. *Indian Farming*, 68, 24–27.
- Khotasena, S., Sanitchon, J., Chankaew, S., & Monkham, T., 2022. The basic vegetative phase and photoperiod sensitivity index as the major criteria for indigenous upland rice production in Thailand under unpredictable conditions. *Agronomy*. 12, 1–12. <https://doi.org/10.3390/agronomy12040957>
- Li, Z., Liang, Y., Yuan, Y., Wang, L., Meng, X., Xiong, G., Zhou, J., Cai, Y., Han, N., Hua, L., Liu, G., Li, J., Wang, Y., 2019. OsBRXL4 regulates shoot gravitropism and rice tiller angle through affecting LAZY1 nuclear localization. *Molecular Plant*. 12, 1143–1156. <https://doi.org/10.1016/j.molp.2019.05.014>
- Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*. 148, 350–382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Mackill, D.J., David J., Coffman, W.R., Garrity, D.P., 1996. *Rainfed Lowland Rice Improvement*. IRRRI, Manila.
- Okuno, A., Hirano, K., Asano, K., Takase, W., Masuda, R., Morinaka, Y., Ueguchi-Tanaka, M., Kitano, H., Matsuoka, M., 2014. New approach to increasing rice lodging resistance and biomass yield through the use of high gibberellin producing varieties. *PLoS ONE*. 9, e86870. <https://doi.org/10.1371/journal.pone.0086870>
- Promchote, P., Wang, S.Y.S., Yoon, J.H., Johnson, P.G., Creech, E., Shen, Y., Yao, M.H., 2022. On the changing cool season affecting rice growth and yield in Taiwan. *Agronomy*. 12, 1–15. <https://doi.org/10.3390/agronomy12112625>
- Sangwongchai, W., Thitisaksakul, M., 2019. The effect of salt stress on productivity, grain morphology, grain carbohydrate and storage protein accumulation of 4 rice (*Oryza sativa* L.) cultivar in degrees of salt tolerance. In: *20th National Graduate Research Conference*. Thailand: Khon kaen University. pp. 459–468.
- Song, Y., Wang, C., Linderholm, H.W., Fu, Y., Cai, W., Xu, J., Zhuang, L., Wu, M., Shi, Y., Wang, G., Chen, D., 2022. The negative impact of increasing temperatures on rice yields in southern China. *Science of the Total Environment*. 820, 1–7. <https://doi.org/10.1016/j.scitotenv.2022.153262>
- Theerawitaya, C., Triwitayakorn, K., Kirdmanee, C., Smith, D.R., Supaibulwatana, K., 2011. Genetic variations associated with salt tolerance detected in mutants of KDML105 (*Oryza sativa* L. spp. indica) rice. *AJCS*. 5, 1475–1480.
- Timeanddate, 2022. Sun Graph for Bangkok. Available at: <https://www.timeanddate.com/sun/thailand/bangkok>. [Date accessed: 13 September 2022]
- Vanavichit, A., Kamolsukyeunyong, W., Siangliw, M., Siangliw, J.L., Traprab, S., Ruengphayak, S., Chaichoompu, E., Saensuk, C., Phuvanartnarubal, E., Toojinda, T., Tragoonrung, S., 2018. Thai hom mali rice: origin and breeding for subsistence rainfed lowland rice system. *Rice*. 11, 1–12. <https://doi.org/10.1186/s12284-018-0212-7>
- Vergara, B.S., Tanaka, A., Lilis, R., Puranabhavung, S., 1966. Relationship between growth duration and grain yield of rice plants. *Soil Science and Plant Nutrition*. 12, 31–39. <https://doi.org/10.1080/00380768.1966.10431180>
- Wickramasinghe, W.M.D.M., Devasinghe, D.A.U.D., Dissanayake, D.M.D., Benaragama, D.I.D.S., Egodawatta, W.C.P., Suriyagoda, L.D.B., 2021. Growth physiology and crop yields of direct-seeded rice under diverse input systems in the dry zone of Sri Lanka. *Tropical Agricultural Research*. 32, 325. <https://doi.org/10.4038/tar.v32i3.8496>
- Widyawati, A.T., Nurbani, Prasetyo, W.B., Manurung, S. E., Pebriadi, A.M., 2021. The effect of rice field conversion and its impact on food availability in North Kalimantan Province. *IOP Conf. Ser.: Earth Environ. Sci.* 824, 012059. <https://doi.org/10.1088/1755-1315/824/1/012059>
- Yan, W., Hu, B., Zhang, Q., Jia, L., Jackson, A., Pan, X., Huang, B., Yan, Z., Deren, C., 2012. Short and erect rice (ser) mutant from “Khao Dawk Mali 105” improves plant architecture. *Plant Breeding*. 131, 282–285. <https://doi.org/10.1111/j.1439-0523.2011.01943.x>
- Zahra, N., al Hinai, M.S., Hafeez, M.B., Rehman, A., Wahid, A., Siddique, K.H.M., Farooq, M., 2022. Regulation of photosynthesis under salt stress and associated tolerance mechanisms. *Plant Physiology and Biochemistry*. 178, 55–69. <https://doi.org/10.1016/j.plaphy.2022.03.003>
- Zhu, L., Chen, Z., Li, H., Sun, Y., Wang, L., Zeng, H., He, Y., 2020. Lipid metabolism is involved in male fertility regulation of the photoperiod- and thermo sensitive genic male sterile rice line Peiai 64S. *Plant Science*. 299, 1–13. <https://doi.org/10.1016/j.plantsci.2020.110581>