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Responses of glutinous rice to water-saving production by alternate wetting and drying technique on physiological and agronomical characteristics in the Northeast of Thailand

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ABSTRACT: The effect of water supply under alternate wetting and drying practice (AWD) on physiological and agronomical characteristics of glutinous rice was investigated in this study. The experiment was conducted at the Faculty of Agriculture, Ubon Ratchathani University during 2021-2022. The experimental design was a 4 x 2 Factorial in completely randomized design (CRD) with 2 factors. Factor A consisted of 4 regimes of water: Continuously flooding (CF) (control), alternate wetting and drying of AWD15, AWD25 and AWD35 where re-flooding was performed when the below-ground water level at 15, 25 and 35 cm, respectively. Factor B comprised of 2 varieties of glutinous rice, Leenok (a local variety) and RD22 (a modern variety). Results showed that the height of the rice plants grown under AWD15 and AWD25 was higher than that of AWD35 at 60 DAT, whereas the highest tiller number per hill of the rice plants grown under AWD25 was found compared with the CF. The rice plants under AWD35 showed longer root length and root to shoot ratio than CF. While, the root dry weight was the lowest. The photosynthetic rate (A) was highest in the rice plants under CF and lowest under AWD35 condition. No significant difference among the treatments was observed for the maximum quantum efficiency of PSII (Fv/Fm), transpiration rate (E), sub-stomatal CO2 concentration (Ci) and the stomatal conductance (Gs). Lower leaf chlorophyll contents were observed for the rice plants under AWD25 and AWD35 compared to CF and AWD15, while the carotenoid content was highest under AWD35. Also, Leeknok had a lower plant height, but a greater number of tillers than RD22. However, there was no significant difference in the physiological responses of both varieties. Results suggested that the water regime at AWD25 seemed to accommodate growth and physiological responses of the glutinous rice better than CF under the growing condition of the Northeast of Thailand.

Keywords: glutinous rice; alternate wetting and drying; rice physiological response; rice growth

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important staple food crops and plays a significant role in world's food security and sustainability as it is consumed by billions of people around the world, especially in Asia (Schneider and Asch, 2020). It is widely cultivated across Asia and other parts of the world with different varieties or subspecies being planted in deferent growing regions (Gadal et al., 2019). Glutinous rice (*Oryza sativa* L. V*ar. glutinosa)*, in particular, is an important food crop for people in the North, Northeast of Thailand and Laos. It is widely consumed as a main staple or turned into other finished products such as snacks, local sweets and desserts.

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As rice cultivation requires a large amount of water, thus water consumption is one of the major limiting factors affecting rice production, especially off-season production using irrigated water. Bouman and Tuong (2001) reported that about 80% of the total irrigated fresh water resources is used by traditional continuous flooding for rice production in Asia. In addition, extreme climate fluctuations dominated by either drought or flooding incidence due to global warming can affect the rice productivity. By using a modeling technique, Horie (2019) projected that global warming caused by the increase in the atmospheric concentration of greenhouse gases would have enormous impact on the uncertainty of future food security in Asia. Therefore, it is important and practical to develop and adopt the water saving technology for rice cultivation to conserve and sustain the available water resources in order to maximize growth and yield to ensure the optimal food supply and sustainability for human population.

The intermittent irrigation by alternate wetting and drying (AWD) practice for rice production has been introduced for many years and shown many beneficial advantages for optimal rice production to achieve high-water productivity (Zhang et al., 2012; Takayoshi et al., 2016; Carrijo et al., 2017; Majeed et al., 2017; Carrijo et al., 2018). The rice plants are capable of adjusting their physiological responses and agronomical traits in order to cope with such conditions (Pandey and Shukla, 2015; Carrijo et al., 2018). However, different rice varieties responded differently to AWD conditions depending on various factors (Kumar et al., 2017), thus, more researches on the impacts of AWD on different rice variety and under different growing areas with different geographical conditions are still needed to obtain more extensive knowledge to improve this water-saving rice production technology. Normally, the glutinous rice is grown in the semi-arid areas in the Northeast of Thailand and Loas with poor soil quality planting conditions. Based on the literature review, no study on the responses of glutinous rice to AWD practice has been reported, thus more research on the effects of AWD practice on growth and physiological responses of the glutinous rice is still required. Results on the glutinous rice's responses under such condition should be useful for the technological adaptation for the glutinous rice production. Therefore, this research aimed at examining the growth and physiological responses of the local and modern variety of the glutinous rice to such water regimes. The data obtained should be useful for a further study to investigate to the effect of this technique on growth and yield of the rice under field condition so that such practice could be adopted for the glutinous rice production in the region.

Materials and methods

Study site information

The experiment was conducted at the Nursery of the Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani, Thailand during the dry season from November 2021-April 2022. The data of soil analysis showed that the soil texture was sandy loam with sand (68.65%), silt (16.85%) and clay (15.38%), pH (6.45), organic matter (0.43 %), avail. P (21.82 ppm), exch. K (89.38 ppm), field capacity (10.90%) and permanent wilting point (6.60). These properties were in line with report by Taparat and Soontorn (2016) for soil property in the Northeast region of Thailand. Meteorological data for the area from the Northeastern Meteorological Station, Ubon Ratchathani Province (2021-22) recorded the average precipitation during the experimental period of 4.62 mm and 14.5-20.5 and 31.1-36.1 °C for the minimum and maximum temperature, respectively, and 63-72% for the relative humidity.

Experimental design

A water-proof cement block with a diameter of 80 cm and a height of 40 cm was used as the experimental plot and a soil mixture of sandy loam soil (with soil property described above) and a cow manure compost mixture (at a ratio of 3:2 w/w) was used as the growing medium for the experiment. Each plot was filled with the soil mixture to the level of 35 cm from the ground and water was added to incubate the soil mixture for 3 weeks before starting the experiment. A factorial design in completely randomized design (CRD) with two factors, water level as Factor A and rice variety as Factor B, was used. For Factor A, four levels of water management regimes were applied, including continuous flooding (CF) by keeping the water level at 5 cm above ground all the time as a control treatment, and three levels of alternate wetting and drying practice (AWD) where the water level was refilled up to 5 cm above ground when the groundwater level reached 15 cm (AWD15), 25 cm (AWD25) and 35 cm (AWD35), respectively. These water management cycles were maintained until the maximum growth (60 DAT). For Factor B, two varieties of glutinous rice, Leenok (a local variety) and RD22 (a modern variety), were used as the tested varieties. Each treatment consisted of 4 replications (one cement block as one replication) with 6 hills of rice plants being planted per plot.

Plant preparation and transplanting

Seeds of Leenok and RD22 rice were obtained from a local farmer and a local government organization, respectively. After soaking in water for 24 hours, the seeds were kept at ambient conditions for another 24 hours to allow germination to proceed before sowing into the seedling tray filled with a soil mixture (3-5 seeds per pit). After 20 days of sowing, strong and healthy seedlings were selected and transplanted into each experimental plot using 25 x 25 cm spacing with one seedling per hill and a total of 6 hills per one plot.

Water level management

The water level in all experimental plots were initially maintained at about 5 cm above the soil surface for 15 days after transplanting (DAT) to allow the seedlings to adapt to the growing condition before starting each treatment as described above. For underground water level observation, the PVC pipe (2.5 inches in diameter) were cut into a length of 20, 30 and 40 cm (40 drilled holes per pipe) and buried into each experimental plot with the top end of the pipes exposed about 5 cm from the topsoil giving the observation of the groundwater level for 15 (AWD15), 25 (AWD25) and 35 cm (AWD35) below ground in the corresponding experimental plots, respectively. When the water decreased to a corresponding depth of each plot, the water was then refilled into the plots to the level of 5 cm above the soil surface. These cycles were repeated and monitored until the rice plants reached the panicle initiation stage (60 DAT). After that, the water levels were maintained to a depth of 5 cm above ground for all the experimental plots until the dough grain stage (90 DAT).

Crop growth measurement

Crop growth data, including plant height (cm), number of tillers per hill, were collected at 15, 30, 45, and 60 DAT. For the data collection on root length, root dry weight and root to shoot ratio, a separate experiment was conducted and the measurement was done at 60 DAT. For root length measurement, the whole plant with the whole root system was carefully removed from the growing medium and the dirt was removed from the root and cleaned by clean water. After air dry for one day, the root length of individual crop plant from each treatment (6 hills per replicate and 4 replications per treatment) was measured. For root dry weight and root to shoot ratio

measurement, the root and shoot samples were separated and dried in the oven at 70 °C for 48 hr and the weight of the samples, before and after drying, was measured by a three-digit digital scale and then the root to shoot ratio was calculated accordingly.

Physiological data collection

Data on physiological responses of the rice leaf were collected at the maximum growth (60 DAT). The 3rd leaf position from top of the main culm of each hill was selected (six replicates per experimental unit) and measured for chlorophyll fluorescence, rate of photosynthesis, transpiration rate, sub-stomatal CO_2 concentration, stomatal conductance and chlorophyll and carotenoid contents. All measurements of all treatments were carried out between 10:00 am to 2:00 pm on the same day as follows:

Chlorophyll fluorescence was measured by using a Plant Efficiency Analyzer (Handy PEA, Hanstech, Japan). The leaf was clipped for 20 min for dark adaptation and the measurement of variable:maximum fluorescence (*Fv/Fm*) (maximum quantum efficiency of PSII) was recorded with full light exposure for 5 seconds (Munns and James, 2003). The photosynthetic rate (A, μmole CO_2 m^{-2} s^{-1}) was measured by using a Portable Photosynthetic System (Li-Cor, LI-1600, USA). The transpiration rate *(E, $\mu mol\ m^{-2}\ s^{-1}$),* sub-stomatal CO_2 concentration (Ci, ppm), stomatal conductance (Gs, cm s^{-1}), were measured by using a Steady State Porometer (Delta-T Device, England).

For quantification of leaf chlorophyll and carotenoid contents, an aliquot of 7 ml of dimethylformamide (DMF, 99.8%) was added to 0.2 g of leaf sample in a test tube and incubated in a water bath at 70°C for 90 minutes before cooling at room temperature. The extract was then filtered with Whatman filter paper #42 and the final volume was brought up to a total of 10 ml with DMF. The absorbance of the sample was measured by a Spectrophotometer (Shimadzu, UV1206, Japan) at the wavelengths of 664.2, 648.6, and 470 nm, consecutively, and calculations for chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll (*Chl* a+b), chlorophyll a/b ratio and carotenoids were done according to Yashida et al. (1981) as follows:

$$\text{Chlorophyll } a = \frac{((12.7(A664) - 2.69(A648)) \times V}{1{,}000 \times W} \text{ mg/g (FW)}$$

$$\text{Chlorophyll } b = \frac{((22.9(A648) - 4.68(A664)) \times V}{1{,}000 \times W} \text{ mg/g (FW)}$$

$$\text{Carotenoid} = \frac{(1{,}000(A470) + 3.27((\text{Chl } a - \text{Chl } b))) \times V}{229 \times W} \text{ g/100 ml}$$

W = Weight samples (g)

V = Solution volumes used (ml)

FW = Fresh weight (g)

A = Wave lengths (nm)

Soil moisture content

The soil sample from each plot was taken for the moisture content analysis at 35, 45 and 60 DAT. About 100 g of wet soil sample was collected from the top soil surface by using a soil auger and subjected to oven drying at 105 ℃ for 24 h. The subsequent dry soil weight was recorded and the moisture content was calculated as followed:

$$\text{Moisture content (\%)} = \frac{\text{(Weight of wet soil sample (g) - Weight of dry soil sample (g))}}{\text{Weight of dry soil sample (g)}} \times 100$$

Water use

The total amount of water added each plot was recorded during the experimental period and calculated for water use (m^3 rai^{-1}).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and means and mean separation and comparison were done by using the least significant (LSD) procedure with 95% confidence level (Gomez and Gomez, 1984) using the SPSS program (Ver 15).

Results

Plant growth

Plant height

The height of the rice plants was highly affected by both the water level and the rice variety during 15, 30, 45 and 60 days after transplanting (DAT) (*p<0.01*) **(Table 1)**. The height of the rice plants grown under CF condition was significantly higher than other treatments at 15, 30 and 45 DAT, followed by AWD15, while the height of those grown under AWD25 and AWD35 was lowest. However, at full growth (60 DAT), the rice plants grown under CF, AWD15 and AWD25 were not significantly different, while that of those grown under AWD35was lowest. Furthermore, for a variety comparison, RD22 had consistently higher plant height than Leenok throughout the experimental period (*p<0.01*). There was also highly significant effect of interaction (*p<0.01*) between the water level and the rice variety on plant height at 15, 30 and 45 DAT, where RD22 rice grown under AWD15 had the highest height, while Leenok rice under AWD25 and AWD35 had the lowest height.

Number of tillers per hill

The number of tillers of the rice plants was also highly influenced by both the water level and the rice variety during 15, 30, 45 and 60 DAT (*p<0.01*) **(Table 1)**. At 15 DAT, the tiller number per hill of the rice plants grown under AWD25 was significantly higher than other treatments, while at 30 DAT, it was followed by CF, AWD15, AWD35, respectively. At 45 DAT, the tiller number per hill of the rice plants under AWD15 and AWD25 was significantly higher than that of those grown under CF and AWD35, whereas, at full growth (60 DAT), the tiller number per hill of the rice plants under CF condition was significantly lower than AWD25. For individual variety, Leenok had a significantly greater number of tillers per hill than RD22 at 30-60 DAT (*p<0.01*). In addition, a highly interaction effect between the water level and the rice variety on the rice tiller number was observed at 30, 45 and 60 DAT (*p<0.01*), where

Leenok variety grown under AWD25 had the highest tiller number per hill, whereas RD22 under AWD25 showed the lowest number of tillers per hill. However, both varieties exhibited similar number of tillers under CF condition.

Table 1 Plant height (cm) and number of tillers per hill of two glutinous rice varieties (Leenok and RD22) at 15, 30, 45 and 60 DAT under different water regimes

Factors	Height (cm)				Number of tillers per hill			
	15	30	45	60	15	30	45	60
A (Water regimes)								
CF	28.00 a	45.16 a	80.59 a	88.36 ab	3.35 b	6.45 b	12.54 b	19.85 b
AWD15	26.45 b	42.84 b	79.21 b	88.79 a	3.48 b	5.98 c	14.59 a	22.27 ab
AWD25	24.93 c	36.49 c	73.80 c	89.65 a	3.95 a	7.44 a	16.39 a	23.85 a
AWD35	25.64 c	35.58 c	72.99 c	86.15 b	3.39 b	5.39 d	10.67 b	21.16 ab
B (Variety)								
Leenok	25.72 b	39.35 b	70.64 b	84.25 b	3.60	6.80 a	14.81 a	23.53 a
RD22	26.78 a	40.68 a	82.66 a	92.23 a	3.49	5.84 b	12.28 b	20.04 b
A x B (Variety)								
Variety 1 (Leenok)								
CF	26.63 b	43.03 b	78.80 d	83.33	3.46	6.46 b	11.21 cd	19.46 bcd
AWD15	26.40 b	42.90 b	74.48 e	83.85	3.51	6.33 b	15.35 b	22.96 bc
AWD25	24.70 d	36.15 c	64.83 f	86.35	3.95	8.80 a	20.95 a	28.62 a
AWD35	25.15 cd	35.33 c	64.45 f	83.48	3.50	5.59 cd	11.75 cd	23.08 b
Variety 2 (RD22)								
CF	29.37 a	47.30 a	82.38 bc	93.40	3.35	6.44 b	13.87 bc	20.25 bcd
AWD15	26.50 b	42.78 b	83.95 a	93.73	3.46	5.63 cd	13.83 bc	21.59 bcd
AWD25	25.15 cd	36.83 c	82.78 ab	92.95	3.95	6.08 bc	11.83 cd	19.08 d
AWD35	26.13 bc	35.83 c	81.27 c	88.83	3.29	5.19 d	9.58 d	19.24 cd
F-Test A	**	**	**	**	**	**	**	*
B	**	**	**	*	ns	**	**	**
A x B	**	**	**	ns	ns	**	**	**
CV (%)	2.73	3.34	1.05	2.83	5.63	6.45	13.85	11.90

Means with different letters in the same column indicate significant difference at *, $P < 0.05$; **, $P < 0.01$; ns, non significant

Root length, root dry weight and root to shoot ratio

A highly significant effect of the water level on root length, root dry weight and root to shoot ratio was observed (*p<0.01*) **(Table 2)**. Root length of the rice plants exposed to water regimes at AWD35 was significantly longer than other treatments but there was no significant difference between the two glutinous rice varieties. However, it was the opposite for the root dry weight where exposure to AWD35 resulted in the lowest root dry weight of the rice plants and the highest root dry weight was found under CF condition. Leenok variety had a significantly greater root dry weight than RD22. For root to shoot ratio, the rice plants grown under AWD35 showed

the highest ratio, followed by AWD25, AWD15 and CF, respectively, and no significant difference between the two varieties was detected. Significant effects of interaction between the water regimes and the rice variety were observed for the root length and the root dry weight but not for the root to shoot ratio. For the root length, both Leenok and RD22 varieties showed the highest root length under AWD35 followed by Leenok rice under AWD25, whereas RD22 rice grown under AWD15 had the lowest the root length. For the root dry weight, both Leenok and RD22 rice grown under CF condition showed the highest values, while RD22 rice grown under AWD25 and AWD35 showed the lowest value.

Table 2 Root length (cm), root dry weight (g) and root to shoot ratio of two glutinous rice varieties (Leenok and RD22) at the maximum growing stage (60 DAT) under different water regimes

Factors	Root length	Root dry weight	Root shoot ratio
	(cm)	(g)	
A (Water regimes)			
CF	22.63 b	23.88 a	0.23 d
AWD 15	21.75 b	18.63 b	0.25 c
AWD 25	23.13 b	18.63 b	0.26 b
AWD 35	28.75 a	16.88 c	0.31 a
B (Variety)			
Leenok	24.50	22.31 a	0.26
RD22	23.63	17.19 b	0.26
A x B			
Variety 1 (Leenok)			
CF	21.75 cd	24.25 a	0.24
AWD15	22.50 bcd	21.50 bc	0.26
AWD25	24.25 b	22.75 abc	0.27
AWD35	29.50 a	21.75 c	0.31
Variety 2 (RD22)			
CF	23.50 bc	23.50 ab	0.24
AWD15	21.00 d	18.75 d	0.25
AWD25	22.00 cd	14.50 e	0.28
AWD35	28.00 a	12.00 e	0.31
F-Test A	**	**	**
B	ns	**	ns
A x B	*	**	ns
CV (%)	5.51	8.58	3.95

Means with different letters in the same column indicate significant difference at *, $P < 0.05$; **, $P < 0.01$; ns, non significant

Plant physiological responses

Chlorophyll fluorescence (ChFl, Fv/Fm)

The chlorophyll fluorescence, expressed as variable:maximal fluorescence ratio (Fv/Fm) indicating the maximum quantum efficiency of PSII, was not influenced by neither the Water regimes nor the rice variety **(Table 3)**. Although the rice plants grown under CF condition showed slightly higher value than other treatments, but it was not significant (*p>0.05*) and both Leenok and RD22 variety showed similar *Fv/Fm* value. Also, the interaction effect between the water regime and the rice variety Fv/Fm fluorescence was not significant.

Photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1})

The photosynthetic rate (A) of the leaf was highly affected by the Water regimes (*p<0.01*), but there was no significant difference among the varieties **(Table 3)**. CF treatment resulted in the highest photosynthetic rate followed by AWD15 and AWD25 treatment, while exposure to AWD35 resulted in the lowest leaf photosynthetic rate of the rice plants. However, the A value of Leenok and RD22 variety was not significantly different and there was on interaction between the water regime and the rice variety on photosynthesis.

Transpiration rate (E, µmol H_2O m^{-2} s^{-1})

The transpiration rate (E) of the rice plants grown under different conditions was not significantly different (*p>0.05*) and there was no significant difference between Leenok and RD22 variety **(Table 3)**. Similarly, the interaction between the water regime and the rice variety on the transpiration rate was not significant (*p>0.05*).

Substomatal CO_2 concentration (Ci, ppm)

Similar to the transpiration rate, the concentration of CO_2 in the substomatal layer of the rice leaf was not affected by neither the Water regimes nor the variety (*p>0.05*) **(Table 3)**. The concentration of CO_2 of the rice plants grown under different Water regimes was not significantly different and both Leenok and RD22 showed a close level of internal CO_2. Also, the interaction water regime and both variety on the Ci concentration was not significant (*p>0.05*).

Stomatal conductance (Gs, cm s^{-1})

The stomatal conductance (Gs) of the rice leaf stomata was not significantly different among the water treatments (*p>0.05*) **(Table 3)**. Both Leenok and RD22 showed similar values and the interaction between the water regime and the rice variety on the Gs value was not significant (*p>0.05*).

Table 3 Chlorophyll fluorescence (ChlFl, Fv/Fm), photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, µmol H_2O m^{-2} s^{-1}), sub-stomatal CO_2 concentration (Ci, ppm), and stomatal conductance (Gs, cm s^{-1}) of the rice leaves at the maximum growing stage (60 DAT) under different water regimes

Factors	ChlFl	A	E	Ci	Gs
	(F_v/F_m)	(µmol CO_2 m^{-2} s^{-1})	(µmol H_2O m^{-2} s^{-1})	(ppm)	(cm s^{-1})
A (Water regimes)					
CF	0.46	13.27 a	8.89	300	0.24
AWD15	0.35	10.85 b	8.92	309	0.23
AWD25	0.34	10.53 bc	8.73	289	0.22
AWD35	0.37	8.99 c	7.38	287	0.22
B (Variety)					
Leenok	0.38	11.55	8.01	292	0.23
RD22	0.38	11.27	7.94	300	0.23
A x B					
Variety 1 (Leenok)					
CF	0.44	12.39	9.11	289	0.25
AWD15	0.36	10.61	8.18	305	0.24
AWD25	0.36	10.74	8.87	283	0.22
AWD35	0.36	8.46	6.91	289	0.21
Variety 2 (RD22)					
CF	0.49	14.15	8.67	310	0.23
AWD15	0.34	11.09	8.66	313	0.22
AWD25	0.33	10.33	8.58	295	0.23
AWD35	0.38	9.51	7.87	284	0.23
F-Test A	ns	**	ns	ns	ns
B	ns	ns	ns	ns	ns
A x B	ns	ns	ns	ns	ns
CV (%)	31.81	13.83	27.54	9.89	18.16

Means with different letters within the same column indicate significant difference at *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Plant photosynthetic pigments

Chlorophyll a (Chl a)

Water regimes imposed highly significant effect on leaf Chl a accumulation (*p<0.01*) **(Table 4).** The amount of Chl a of the rice plants grown under AWD25 and AWD35 was very significantly lower than those grown under CF and AWD15 conditions (*p<0.01*). However, there was no significant difference between Leenok and RD22 variety and the interaction effect of both factors (water regime and variety) on Chl a content was not significant (*p>0.05*).

Chlorophyll b (Chl b)

Leaf Chl b content was also significantly affected by the Water regimes (*p<0.01*) **(Table 4).** The rice plants grown under CF and AWD15 contained the highest leaf Chl b content followed by AWD25, while those grown under AWD35 contained the lowest leaf Chl b content. However, the Chl b content between Leenok and RD22 variety was not significantly different. Also, the interaction effect of the water regime and rice variety on leaf Chl b content was not significant (*p>0.05*).

Total Chlorophyll (Chl a+b)

The content of total chlorophyll (Chl a+b) was highly affected by the Water regimes (*p<0.01*) **(Table 4).** Under CF and AWD15 condition, the rice plants showed higher accumulation of total chlorophyll than those under AWD25 and AWD35 Water regimes. However, the total chlorophyll of both Leenok and RD22 variety was not significantly different and the interaction effect between the water regime and the rice variety was not significant (*p>0.05*).

Chlorophyll a/b ratio

The ratio of Chlorophyll a/b was also highly influenced by the Water regimes (*p<0.01*) with CF and AWD15 Water regimes gave the highest Chlorophyll a/b ratio, while AWD25 and AWD35 Water regimes showed the lowest Chlorophyll a/b ratio **(Table 4)**. There was no significant difference between Leeknok and RD22 variety but there was a significant interaction effect between the water regime and the rice variety on Chlorophyll a/b ratio (*p<0.05*). Leenok rice leaves contained the highest value under CF condition, whereas Leenok rice under AWD35 showed the lowest value. In contrast, the ratios of Chlorophyll a/b of RD22 rice were not significantly different under all water regimes.

Carotenoids

For leaf carotenoid content, the effect of the Water regimes was obvious and in the opposite direction of the chlorophyll contents. The rice plants grown under severe water deficit (AWD35) showed the highest leaf carotenoid content, whereas the other treatments showed significantly lower leaf carotenoids (*p<0.01*) **(Table 4)**. However, there was no significant difference between the two varieties, and also the interaction effect between the water regime and the rice variety on the leaf carotenoid content was not significant.

Table 4 Leaf **c**hlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a+b (Chl a+b), chlorophyll a/b ratio (Chl a/b) and carotenoids of two glutinous rice varieties (Leenok and RD22) at the maximum growing stage (60 DAT) under different water regimes

Factors	Leaf **c**hlorophyll and carotenoids (mg/g fw)				
	Chl a	Chl b	Chl a+b	Chl a/b	Carotenoids
A (Water regimes)					
CF	1.69 a	1.02 ab	2.72 a	1.73 a	0.59 b
AWD15	1.59 a	1.11 a	2.71 a	1.45 a	0.59 b
AWD25	0.84 b	0.88 bc	1.73 b	0.96 b	0.75 b
AWD35	0.66 b	0.71 c	1.39 b	0.98 b	1.02 a
B (Variety)					
Leenok	1.19	0.90	2.17	1.34	0.74
RD22	1.21	0.96	2.09	1.22	0.74
A x B					
Variety 1 (Leenok)					
CF	1.88	0.92	2.81	2.15 a	0.65
AWD15	1.46	1.05	2.52	1.39 bc	0.55
AWD25	0.84	0.90	1.75	0.94 bc	0.73
AWD35	0.57	0.72	1.30	0.87 c	1.02
Variety 2 (RD22)					
CF	1.50	1.12	2.62	1.31 bc	0.52
AWD15	1.73	1.17	2.89	1.51 b	0.64
AWD25	0.84	0.86	1.71	0.98 bc	0.77
AWD35	0.76	0.71	1.47	1.09 bc	1.02
F-Test A	**	**	**	**	**
B	ns	ns	ns	ns	ns
A x B	ns	ns	ns	*	ns
CV (%)	33.44	22.36	24.44	31.51	23.77

Means with different letters within the same column indicate significant difference at *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Correlation analysis of physiological parameters

Pearson's correlation analysis of all measured physiological parameters revealed both positive and negative correlations between the parameters as shown in **Table 5**. It was found that the photosynthetic rate (A) showed significantly positive correlation with total chlorophyll (Chl a+b) (*p<0.05*) and was highly correlated with the stomatal conductance (Gs) (*p<0.01*), while it showed highly negative correlation with carotenoid content of the leaf (*p<0.01*). In addition, leaf chlorophyll a content (Chl a) was highly correlated with chlorophyll b (Chl b), total chlorophyll (Chl a+b) and chlorophyll a:b ratio (Chl a/b) (*p<0.01*), while it was significantly correlated the stomatal conductance (Gs)

(*p<0.05*). Furthermore, chlorophyll b (Chl b) showed highly positive correlation with total chlorophyll (Chl a+b) (*p<0.01*), and chlorophyll a:b ratio (Chl a/b) was also highly correlated with the stomatal conductance (Gs) (*p<0.01*). A highly significant negative correlation (*p<0.01*) was found between the leaf carotenoid content and the sub-stomatal CO_2 concentration (Ci) and the stomatal conductance (Gs), and between the transpiration rate (E) and the sub-stomatal CO_2 concentration (Ci). No correlation was found between the maximum quantum efficiency of PSII (Fv/Fm) and other physiological parameters.

Table 5 Pearson's correlation analysis of physiological parameters of two glutinous rice varieties under different water regimes

	Physiological Parameters								
	A	Chl a	Chl b	Chl a+b	Chl a/b	Carotenoid	E	Ci	Gs
A									
Chl a	0.30								
Chl b	0.33	0.62 **							
Chl a+b	0.33 *	0.96 **	0.79 **						
Chl a/b	0.22	0.79 **	0.05	0.63 **					
Carotenoid	-0.65 **	-0.18	-0.29	-0.23	-0.13				
E	0.08	0.29	0.28	0.31	0.09	0.18			
Ci	0.20	-0.04	-0.12	-0.07	0.10	-0.45 **	-0.77 **		
Gs	0.45 **	0.36 *	0.15	0.33	0.42 **	-0.47 **	0.08	0.17	
Fv/Fm	0.16	0.17	0.27	0.22	-0.00	-0.12	0.12	-0.09	-0.16

Soil moisture content (%)

The moisture content of the soil was highly affected by the water management regimes at 30, 45 and 60 DAT (*p<0.01*) **(Table 6)**. CF treatment consistently showed the highest soil moisture content, followed by AWD15, AWD25 and AWD35. However, there was no interaction of both factors (water regime and rice variety) on the soil moisture content.

Water use (m^3 rai^{-1})

Water use, measured by the amount water used per area of production (m^3 rai^{-1}), under different water management regimes showed a highly significant difference (*p<0.01*) **(Table 6)**. CF treatment used the highest amount of water for production as expected, followed by AWD15 and AWD25 water treatment, whereas AWD35 water treatment consumed the least water. In comparison with CF treatment, the relative water uses of AWD15, AWD25 and AWD35 were about 31.23, 33.06 and 42.49% less than CF, respectively. However, there was no significant difference between the rice variety and the interaction of both factors (water regime and rice variety) on water use was not significantly different (*p>0.05*).

Table 6 Shows soil moisture content (%) at 30, 45 and 60 DAT and water use (m^3 rai^{-1}) and water use efficiency (WUE, kg m^{-3}) at 60 DAT of two glutinous rice varieties (Leenok and RD22) under different water regimes

Factors	Soil moisture (%)			Water use
	30	45	60	(m^3 rai^{-1})
A (Water regimes)				
CF	84.38 a	84.00 a	84.75 a	2184 a
AWD15	46.75 b	35.88 b	45.13 b	1502 b
AWD25	9.50 c	11.38 c	11.88 c	1462 b
AWD35	6.25 d	6.63 d	6.13 d	1256 c
B (Varieties)				
Leenok	37.31	35.06	37.38	1613
RD22	36.13	33.88	36.56	1589
A x B				
Variety 1 (Leenok)				
CF	84.00	84.50	85.50	2215
AWD15	47.50	36.75	45.75	1483
AWD25	11.25	12.25	12.00	1472
AWD35	6.50	6.75	6.25	1283
Variety 2 (RD22)				
CF	84.75	83.50	84.00	2153
AWD15	46.00	35.00	44.50	1522
AWD25	7.75	10.50	11.75	1452
AWD35	6.00	6.50	6.00	1228
F-Test A	**	**	**	**
B	ns	ns	ns	ns
A x B	ns	ns	ns	ns
CV (%)	7.12	7.22	7.54	2.78

Means with different letters within the same column indicate significant difference at *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Discussion

Water is an indispensable factor in the growth and productivity of the rice plants. Although rice is not an aquatic plant, but the rice plants can germinate and grow in both under water-logged conditions and optimum soil moisture. In this study, it was found that growth of the rice plants under CF increased relatively faster than other treatments during the first 45 DAT where the height of the rice plants grown under CF condition with continuing Water regimes at 5 cm above ground showed significantly higher than other treatments and those under the AWD25 and AWD35 showed significantly lower plant height during the same period **(Table 1)**. However, at 60 DAT, the height of the rice plants grown under CF, AWD15 and AWD25 were not significantly different whereas that of the

AWD35 was lowest. This result indicated that water regime at AWD15 and AWD25 the glutinous rice plants could adapt to these water regimes and exhibit similar growth to CF condition at the full growth period even though relatively slow growth was observed at the initial growth stage (15-45 DAT). On the other hand, the water regime at AWD35 significantly reduced the plant height at full growth (60 DAT). For a variety comparison, the height of RD22 was consistently greater than that of Leenok variety throughout the growing period, indicating the characteristic of these two varieties where the modern variety (RD22) was taller than the local variety (Leenok). In addition, both the water regimes and the varieties exerted the significant effect on the plant height as the interaction analysis of both factors was highly significant ($p<0.01$), where the highest plant height was found in RD22 rice grown under AWD15 and the lowest plant height was found in Leenok rice under AWD25 and AWD35. This suggested that both varieties responded differently to different water management regimes showing that RD22 rice seemed to perform better than Leenok rice at the early stage of growth. Hoever, there was no interaction effect of both factors (water regimes and varieties) on plant height at the maximum growth (60DAT).

In contrast, the impact of the water regime on the tiller number was quite opposite of the plant height where the glutinous rice plants grown under the AWD25 showed consistently greater number of tillers than those under CF condition throughout the growing period, while those grown under AWD15 and AWD35 were in between **(Table1)**. These results suggested that these two glutinous rice varieties performed well under wetting and drying cycle of the AWD technology in term of tillering capacity and did not require continuous Water regimes all the time to produce better tiller number. In addition, Leenok showed consistently higher number of tillers than RD22 throughout the growing period. These results collectively distinguished the attributes of the two varieties that the modern variety (RD22) was taller but produced lower tiller number than the local variety (Leenok). The significant interaction effect on the number of tillers per hill ($p<0.01$) was observed showing that Leenok rice produced the highest, while RD22 rice produced the lowest tiller number per hill under AWD25. This result indicated that Leenok rice showed better tillering capacity than RD22 rice under AWD25, which may imply better ability to adapt to a mild water deficit condition for rice production.

In addition, for root length, the water regime at AWD35 caused the root to grow deeper as indicated by the longest root length of the rice plant under AWD35 condition and similar result was found for root to shoot ratio **(Table 2)**. In contrast, the root dry weight of the rice plants grown under CF condition was the highest compared to other treatments **(Table 2)**. The interaction effect showed that both Leenok and RD22 varieties had the highest root length under AWD35, whereas RD22 rice grown under AWD15 had the lowest the root length. Moreover, both rice varieties showed the highest root dry weight under CF condition, while RD22 rice showed the lowest value under AWD25 and AWD35. These results suggested that the always abundance of water under CF growing condition led to the highest root dry mass accumulation of the rice plants but the lowest root to shoot ratio, whereas the water regime at AWD35 rendered the longest root length and the highest root to shoot ratio, but the lowest root dry weight. These may imply the physiological adaptation of the root system of the glutinous rice plants in response to the water regimes that affirmed the common phenomenon of root growth in response to water or moisture availability of the plant. Although there was no significant difference between Leenok and RD22 varieties on the root length and the root to shoot ratio, but Leenok variety showed greater root dry weight than RD22 variety.

Analysis of the physiological data of the rice leaves at the maximum growth (60 DAT) under different Water regimes conditions revealed that only significant difference was observed for the photosynthetic rate (A), whereas there was no significant effect of the water management regimes on the maximum quantum efficiency of PSII (Fv/Fm), transpiration rate (E), sub-stomatal CO_2 concentration (Ci), and stomatal conductance (Gs) of the leaf **(Table 3)**. The photosynthetic rate of the rice plants under CF condition was highest, while that of the rice plants under water regime at AWD35 was lowest. Moreover, the rice plants grown under the AWD15and AWD25 water regimes seemed to have lower photosynthetic rate than those under CF condition, but the plant height and root growth were similar. Also, lower number of tillers was observed under CF condition. This indicated that the AWD35 imposed the significant impact on the photosynthetic capacity of the glutinous rice plants leading the lowest growth under such condition **(Table 3)**. In addition, the impact of the water regimes on the photosynthetic pigment contents was significant where the rice plants under the AWD25 and severe (AWD35) Water regimes had lower contents of chlorophyll a, chlorophyll b, total chlorophyll (a+b) and chlorophyll a/b than those under CF and AWD15 conditions **(Table 4).** In contrast, the carotenoid content was the opposite where the AWD35 condition led to the highest accumulation of carotenoids in the leaves of the rice plants. This implied that carotenoids played an important role in protecting the photosynthetic apparatus under the AWD35 condition. The carotenoid content in the rice leaves was highest under the AWD35 coinciding with the lowest photosynthetic rate and the lowest plant height of the rice plants under such condition.

Both positive and negative correlations among the physiological parameters revealed the relationship of the physiological responses with the photosynthetic pigments **(Table 3)**. The photosynthetic rate was positively correlated with the total leaf chlorophyll content and stomal conductance (Gs), while it was negatively correlated with leaf carotenoids, indicating that the photosynthesis of the glutinous rice was more dependent on the photosynthetic pigments (total chlorophylls) and stomal conductivity. On the other hands, the negative correlation was found between the leaf carotenoid content and the sub-stomatal CO_2 concentration (Ci) and the stomatal conductance (Gs), and between the transpiration rate (E) and the sub-stomatal CO_2 concentration (Ci), whereas no correlation was found between the maximum quantum efficiency of PSII (Fv/Fm) and other physiological parameters. Although the quantum or fluorescence yield (Fv/Fm) was not different among the treatments indicating that the efficiency of photosynthesis did not alter under such condition as the rice plants might be able to cope with the prolonged water deficit and resume normal activity after receiving the water after the drying cycle (Yoshida, 1981; Ishihara and Saito, 1983; Zhu et al., 2017; Xu et al., 2020). Severe root dehydration reduced the water potential (WP) of the leaves and closed stomata. This leads to a reduction in the photosensitivity of PS II, accumulation of ABA, an increase in reactive oxygen species such as hydroxyl radical (OH) and superoxide radical (O_2) that can attack DNA, RNA, or proteins, causing the cells and other related mechanisms to alter (Ishihara and Saito, 1983; Zhu et al., 2017; Rodrigues et al., 2019; Ramegowda et al., 2020; Xu et al., 2020; Zhu et al., 2020).

Under CF and water regime at AWD15, both varieties of the glutinous rice (Leenok and RD22) were able to undergo their normal growth and physiological responses, indicating that the roots of the rice plants received enough water and enough light to open up the stomata and fix the atmospheric CO_2 for photosynthesis to synthesize sugars used for growth (Yang et al., 2017). Thus, the efficiency of light used and photosynthesis of the rice plants did not decrease, which sufficiently supported the growth and tillering capacity of the rice plants (Rahman and Bulbul, 2014;

Majeed et al., 2017). In contrast, when the groundwater was reduced to the AWD25, the dehydration of the rice roots was not severe and stressful, and the reduced contents of chlorophyll a and b did not greatly affect the physiological growth of the rice with slight decrease in the efficiency of light used and photosynthesis of the rice plants, but it was sufficient to accommodate the suitable plant growth and tillering capacity under this condition. However, when the water regime at the AWD35, the roots were severely affected and the chlorophyll contents decreased as the carotenoid level increased, affecting the physiological response and growths of the rice plants.

The significant alteration of the soil under AWD practice was the difference in the soil moisture content as it was highly affected by the water management regimes ($p<0.01$) **(Table 6).** CF treatment consistently showed the highest soil moisture content as expected, followed by AWD15, AWD25 and AWD35. This would definitely affect the physiological and growth responses of the rice plants under such conditions as reported in this study. Furthermore, one of the most prominent benefits of the AWD water management practice is the water-saving attribute for rice production, which becomes increasing more important for the current situation as the climate change becomes more erratic and unpredictable. In this study, it was found that AWD15, AWD25 and AWD35 used about 68.77, 66.94 and 57.51%, respectively, compared to the water use by CF practice. Thus, this water-saving ability should be useful for future glutinous rice production, especially off-season production. However, a further study should be conducted to evaluate the effect of this water management regime on yield and yield components as well as grain quality under field condition in order to adapt such practice for glutinous rice cultivation in the Northeast of Thailand.

Based on the results of this experiment, it can be assumed that high chlorophyll contents did not always lead to higher photosynthetic capacity and greater growth as the rice plants grown under the water regime at AWD25 with lower photosynthetic rate and lower chlorophyll contents showed similar growth to those grown under CF condition. Furthermore, water regime at AWD35 led to the lowest growth, lowest photosynthetic rate, lower chlorophyll contents and the highest carotenoid accumulation in the leaves, indicating that under such condition the rice plants lost the adaptation ability to cope with the water shortage. Thus, it can be concluded that suitable water regime allowed the photosynthetic apparatus to have more capacity to absorb carbon dioxide and photosynthetic rate to sustain growth of the rice plants, but when the groundwater reached the critical level (AWD35), it affected both growth and physiological responses leading to altered growth of the rice plants.

Conclusions

In conclusion, when considering the plant growth, the height of the rice plants grown under CF and water regimes at AWD15 and AWD25 conditions was not significantly different, while the tiller number of the rice plants grown under the water regime at AWD25 was greater than CF. Together with a lower water consumption, the water regime at AWD25 seemed to be more suitable for the glutinous rice production than CF. As correlation analysis revealed both positive and negative correlation, showing that the relationship between the leaf photosynthesis and photosynthetic pigments was closely related under different water shortage conditions. The glutinous rice plants used such mechanism to cope with the water shortage. In addition, in term of a variety selection, based on the result of this study it was found that the overall performance of both varieties was quite similar except that the local variety (Leenok) seemed to have a better tillering capacity than the modern variety (RD22), which would give rise to higher yield under alternate wetting and drying (AWD) rice production technology. However, this study did

not include yield and yield component analysis, which would be included in another subsequent study to further investigate the effect of CF and the moisture regime at AWD25 on growth, yield and yield components, and other physiological responses of Leenok and RD22 glutinous rice varieties under field conditions.

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