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Effects of hydrogen peroxide application on agronomic traits of rice (*Oryza sativa* L.) under drought stress

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Abstract: Drought stress is a major environmental factor limiting crop growth and productivity. Hydrogen peroxide (H_2O_2) plays an essential role during stress response by acting as a signal molecule that activates multiple stress tolerance mechanisms. In this study, the effects of H_2O_2 on agronomic traits were studied in rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105 (KDML 105) was subjected to drought stress. H_2O_2 was applied by either seed priming or foliar application method with a concentration of 1, 5, and 15 mmol/L. The results showed that both seed priming and foliar application with H_2O_2 improved some yield components. The tiller numbers, number of panicles, number of filled grains, filled grain weight, and harvest index were improved approximately 1.13, 1.04, 1.23, 1.21, and 1.1 times compared to the untreated plants. Foliar application, however, helps the plant by reducing yield loss as indicated by a 0.5-time reduction in the number of unfilled grain and lower unfilled grain weight. It was suggested that 5 mmol/L H_2O_2 was the most effective concentration to alleviate the effect of drought stress during the reproductive stage in rice.

Keywords: abiotic stress; drought tolerance; foliar spray; rainfall; antioxidant enzyme; stress acclimation

Drought is considered one of the most severe environmental factors limiting crop growth and productivity. Rice cultivation in many areas of Thailand still largely depends on rainfall, and drought has become a significant problem affecting its yield. The previous study suggested that drought strongly influences rice growth and development, particularly in the reproductive stage (Singh et al. 2018). Zhang et al. (2018) conducted a meta-analysis of 55 published studies and found that drought decreased the agronomic traits by 25.4% among varying growth stages. Besides, rice yield decreased by 53–92% and 48–94% during exposure to mild and severe drought stress (Lafitte et al. 2007). For example, Yang et al. (2019) reported that rice physiological traits and yield decreased by 23.2–24% during the flowering stage. Approximately 67% of Thailand's total rice-growing area is accounted for rain-fed cultivation, especially in the northeastern region of Thailand, and the lowest average grain yield was reported in this area (Jongdee et al. 2006, Haefele and Konboon 2009).

Under unfavourable conditions, plants produce excess amount reactive oxygen species (ROS), which causes peroxidation of lipids, denaturation of proteins, DNA mutation, disruption of cellular homeostasis, and various types of cellular oxidative damage (Pandey and Shukla 2015). Among ROS, hydrogen peroxide (H_2O_2) also plays an essential role in many developmental stages such as germination, growth, senescence, and signaling response to stresses (Černý et al. 2018). H_2O_2 has been used to improve plants' tolerance against various environmental stresses. Seed priming and foliar spray methods are standard methods for H_2O_2 application due to their simplicity and inexpensiveness. Using H_2O_2 as a priming agent in wheat resulted in increased growth, water potential, osmolytes accumulation, antioxidant system, and higher yield under drought stress (Habib et al. 2020). Similarly, foliar application with low concentrations of H_2O_2 helped protect yield loss in cotton by increasing antioxidant enzyme activities (Sarwar

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et al. 2017). The beneficial effects of H₂O₂ were also reported in wheat in which 1 000-grain weight, grain per spike, and some physiological traits were improved under drought stress (Farooq et al. 2017).

Most studies on the effects of H₂O₂ application were performed in the vegetative stage; only a few studies have focused on the reproductive stage. In addition, no data has been reported on the comparison between seed priming and foliar application method under drought stress. Therefore, it is interesting to determine the effects of H₂O₂ on agronomic traits in rice and compare the results between seed priming and foliar application method. The knowledge gained may improve rice productivity by alleviating the effect of drought stress during the reproductive stage.

MATERIAL AND METHODS

Plant materials and growing conditions. Rice cultivation was performed during August–November 2019 at the greenhouse at the Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand. The average humidity during August to November 2019 was 72.45, 74.66, 65.95, and 62.56%, respectively. The average temperature during August to November 2019 was 29.72, 29.07, 29.59, and 27.23 °C, respectively. The experimental design was carried out using a completely randomised design (CRD) with five replicates in each treatment. The experiment was divided into two groups of H₂O₂ application methods, including seed priming and foliar spray. For seed priming treatment, 200 rice seeds were fully immersed in 200 mL in priming solutions, including 1 mmol/L H₂O₂ (SP1), 5 mmol/L H₂O₂ (SP5), and 15 mmol/L H₂O₂ (SP15) at a temperature of 25 °C for 24 h in dark condition. For control, untreated plants, and foliar spray application, the seeds were fully immersed in distilled water. The treated and untreated seeds were then germinated and sown in 20.32 cm plastic pot containing 4.5 kg soil (loamy sand; pH 6.04; organic matter 0.24%; electrical conductivity 0.04 dS/m; total nitrogen 0.02%; total phosphorus 36.5 mg/kg; total potassium 232.5 mg/kg and cation exchange capacity 3.94 cmol₊/kg). Two rice seeds were sown in each pot and grown until the plant reached the developmental stage of phase 14–15 on the BBCH scale (21 days). After that, one vigor seedling was selected, while the other was removed and continued to grow until BBCH 45–47 phase (70 days). Before subjected to drought stress, the plants in the foliar spray group were treated with different

concentrations of H₂O₂, including 1 mmol/L (FP1), 5 mmol/L (FP5), and 15 mmol/L (FP15). In contrast, the control and untreated groups were sprayed with distilled water. The plants were sprayed (20 mL/spray/pot) to a runoff in early morning (8.00 a.m.) and repeated for two days. On day 72 of cultivation, the plants were all exposed to drought stress by reducing irrigation by 25% of the field capacity every two weeks except the control group, which was watered daily. Rice yield was recorded at 120 days after planting.

Plant growth and yield component measurements. Plant height was recorded at day 30 (BBCH 14–15), 60 (BBCH 29–30), 72 (BBCH 45–47), and day 120 (BBCH 99) after planting. For biomass measurement, the plants were separated into the root and the shoot parts. Shoot length, root length and dry weight (DW) of shoot and root were recorded. For dry weight, the samples were dried in a hot air oven at 80 °C for three days, and the weight of each sample was recorded.

At harvesting, yield components were recorded, including the number of tillers/hill and the number of panicles/hill. Panicle length was taken from the base of the rachis to the tip of each panicle. The total number of (filled and unfilled) grains/panicle and hill were manually counted. Filled and unfilled grains were determined by floating the seeds in the water (the submerged grains defined as filled grains) (Zhang et al. 2015). Filled and unfilled weight per panicle, 1 000-grain weight were measured. The harvest index was calculated according to the following formula: harvest index (%) = grain yield × 100/biological yield (Farooq et al. 2017).

Statistical analysis. Statistical analysis using one-way ANOVA and all means were separated at the $P < 0.05$ level using Duncan's multiple range test. Pearson correlation coefficients were calculated to determine the relationship among all parameters.

RESULTS

Growth parameters. In this study, plants on days 30, 60, 72 were not subjected to drought stress, but the data is also reported. The results showed that seed priming with H₂O₂ significantly increased plant height during both unstressed and drought conditions. On day 30, the data showed that seed priming with 1 and 5 mmol/L H₂O₂ (SP1 and SP5) significantly increased plant height by 11–13% compared to the control and untreated group. On days 60 and 72,

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seed priming with 5 and 15 mmol/L (SP5 and SP15) also increased plant height by approximately 7–13% compared to the control plant. In the foliar-spray group, all treatments were previously primed with distilled water; therefore, no significant difference was observed during the well-watered period (days 30, 60, and 72) (Table 1).

The plants were subjected to drought stress at day 72 to 120. At day 120, seed priming with all H₂O₂ increased plant height compared to the control and untreated plants, especially when primed with 15 mmol/L H₂O₂. With this concentration, the plant height was improved by 12.10% and 6.89% compared to the control and untreated groups. Foliar-sprayed with H₂O₂, however, did not enhance plant height as observed in seed priming treatment. Plant height was slightly reduced when sprayed with 5 and 15 mmol/L H₂O₂ (Table 1).

The dry weight of both root and shoot parts was dramatically decreased after exposure to drought stress. Root dry weight in the untreated group was reduced by 56.93% compared to the control plant, which was watered daily. On the contrary, shoot dry weight was not much affected by drought stress, as its level was not significantly different between the control and untreated group. Treating with H₂O₂, both seed priming and foliar application, had no effect on root and shoot dry weights compared to the untreated group (Table 1).

Yield components. Drought stress significantly reduced yield, as indicated by a 23.26% reduction in the number of tillers/hill in the untreated group compared to the control plants. Application of H₂O₂

with both methods slightly increased the number of tillers/hill on average of 14.14% in all concentrations compared to the untreated group, although the numbers were not significantly different (Table 2). Drought also reduced the number of total panicles/hill by 14.28% when compared to the unstressed plant. While seed priming with H₂O₂ did not positively affect, the number of panicles/hill, foliar-spray with H₂O₂ seems to help by increasing the number of panicles/hill, especially at high concentrations (Table 2).

Panicle length and the number of primary branches/panicle were not affected when the plant was under drought stress. Similarly, H₂O₂ application also had no effect on both the panicle length and the number of primary branches/panicle in all concentrations (Table 2). Harvest index (HI) was calculated from grain yield per dry weight. The results showed that HI significantly decreased by 36.81% in the stressed plant compared to the control plant. Seed priming with 5 and 15 mmol/L H₂O₂ significantly increased HI by 37.27% and 30.67% compared to the untreated plant, as well as foliar spray with 5 mmol/L H₂O₂, which increased by 39.40% (Table 2).

When the rice plant was subjected to drought stress, the number of filled grains/panicle and the number of filled grains/hill were significantly reduced by 35.77% and 51.45% compared to control, respectively. Seed priming with H₂O₂ resulted in a higher number of filled grains/panicle and the number of filled grains/hill, especially those primed with 5 mmol/L H₂O₂, which increase the number of filled grains/panicle and the number of filled grains/

Table 1. Growth parameters of rice cv. KDML 105 grown after exposure to drought stress

Treatment	Plant height (cm)				Dry weight (g)	
	day 30	day 60	day 72	day 120	root	shoot
Control	84.02 ± 5.05 ^a	120.88 ± 2.53 ^a	153.98 ± 2.52 ^{ab}	174.32 ± 2.54 ^{abc}	93.80 ± 12.60 ^c	36.94 ± 0.97 ^b
Untreated	82.44 ± 2.55 ^a	130.14 ± 3.07 ^{ab}	163.36 ± 8.76 ^{abc}	182.82 ± 2.89 ^{bcd}	40.40 ± 7.60 ^{ab}	34.23 ± 0.71 ^{ab}
SP1	93.58 ± 1.35 ^b	127.72 ± 4.17 ^{ab}	167.54 ± 5.80 ^{bc}	188.16 ± 4.59 ^{cd}	47.75 ± 5.00 ^{ab}	35.87 ± 0.64 ^{ab}
SP5	93.34 ± 1.82 ^b	130.16 ± 5.68 ^{ab}	174.52 ± 6.74 ^c	186.86 ± 5.83 ^{cd}	32.88 ± 2.42 ^{ab}	33.13 ± 1.15 ^{ab}
SP15	89.04 ± 1.45 ^{ab}	135.18 ± 1.71 ^b	175.06 ± 4.85 ^c	195.42 ± 6.19 ^d	25.56 ± 2.85 ^a	32.26 ± 1.07 ^{ab}
FP1	89.04 ± 2.33 ^{ab}	136.18 ± 1.92 ^b	172.76 ± 3.63 ^{bc}	184.16 ± 2.22 ^{bcd}	37.03 ± 2.56 ^{ab}	31.69 ± 2.71 ^a
FP5	94.24 ± 1.93 ^b	128.68 ± 5.14 ^{ab}	154.90 ± 8.23 ^{ab}	169.74 ± 6.83 ^{ab}	39.95 ± 5.00 ^{ab}	32.31 ± 0.99 ^{ab}
FP15	89.98 ± 3.86 ^{ab}	124.42 ± 3.96 ^{ab}	148.12 ± 5.33 ^a	162.22 ± 5.22 ^a	48.92 ± 11.68 ^b	32.64 ± 2.13 ^{ab}

The plants were watered daily from day 0 to day 72 and were exposed to drought stress from day 72 to day 120. Data (means ± standard error, $n = 5$) followed with different letters within the same column indicate a significant difference according to Duncan's multiple range test ($P < 0.05$). SP1 – 1 mmol/L seed priming; SP5 – 5 mmol/L seed priming; SP15 – 15 mmol/L seed priming; FP1 – 1 mmol/L foliar spray; FP5 – 5 mmol/L foliar spray; FP15 – 15 mmol/L foliar spray

Table 2. Yield component of rice cv. KDML 105 grown after exposed to drought stress

Treatment	No. of tillers/hill	No. of total panicle/hill	Panicle length/panicle (cm)	Number of primary branch/panicle	Harvest index (%)
Control	8.60 ± 0.40 ^b	9.80 ± 0.20 ^{bc}	34.88 ± 0.32 ^{ab}	10.74 ± 0.37 ^a	31.89 ± 0.60 ^e
Untreated	6.60 ± 0.40 ^a	8.40 ± 0.68 ^{ab}	34.99 ± 0.71 ^{ab}	11.46 ± 0.20 ^{ab}	20.15 ± 1.52 ^{ab}
SP1	8.20 ± 0.20 ^{ab}	8.60 ± 0.40 ^{abc}	35.39 ± 0.67 ^{ab}	11.15 ± 0.14 ^{ab}	21.47 ± 1.65 ^{abc}
SP5	7.60 ± 0.68 ^{ab}	7.80 ± 0.66 ^a	36.68 ± 0.33 ^b	11.24 ± 0.24 ^{ab}	27.66 ± 2.54 ^{cde}
SP15	7.20 ± 0.20 ^{ab}	7.80 ± 0.37 ^a	35.17 ± 0.60 ^{ab}	11.08 ± 0.17 ^{ab}	26.33 ± 1.96 ^{bcde}
FP1	6.80 ± 0.58 ^{ab}	8.80 ± 0.37 ^{abc}	35.96 ± 0.43 ^{ab}	11.47 ± 0.13 ^{ab}	24.95 ± 2.55 ^{abcd}
FP5	8.00 ± 0.84 ^{ab}	9.40 ± 0.60 ^{bc}	35.84 ± 1.11 ^{ab}	11.72 ± 0.44 ^b	28.09 ± 1.78 ^{de}
FP15	7.40 ± 0.75 ^{ab}	10.00 ± 0.32 ^c	33.86 ± 0.57 ^a	11.29 ± 0.24 ^{ab}	19.44 ± 2.94 ^a

Data (means ± standard error, $n = 5$) followed with different letters within the same column indicate a significant difference according to Duncan's multiple range test ($P < 0.05$). SP1 – 1 mmol/L seed priming; SP5 – 5 mmol/L seed priming; SP15 – 15 mmol/L seed priming; FP1 – 1 mmol/L foliar spray; FP5 – 5 mmol/L foliar spray; FP15 – 15 mmol/L foliar spray

hill by 31.58% and 50.11%, respectively. Foliar spray with 5 mmol/L H_2O_2 also improved the number of filled grains/panicle by 28.28% and 50.29% in the number of filled grain/hill compared to the untreated plant (Figure 1A, B).

On the contrary, drought stress caused significant increases in the number of unfilled grains/panicle by 56.61% and a slight increase in the number of unfilled grains/hill by 17.56%. While seed priming with H_2O_2 alleviated the effect of drought by decreasing the number of unfilled grains/panicle and the number of unfilled grains/hill, the result was more prominent in the plant foliar-sprayed with H_2O_2 at all concentrations. Foliar application with H_2O_2 decreased the number of unfilled grains/panicle by 48–57%, as well as the number of unfilled grains/hill, which was reduced by 38–51% (Figure 1C, D).

Filled grain weight/panicle and filled grain weight/hill were dramatically decreased by 37.10% and 52.27%, respectively, when the rice plant was subjected to drought stress. Seed priming and foliar-spray with H_2O_2 slightly increased the filled grain weight/panicle in all concentrations compared to the untreated plant, except those sprayed with 15 mmol/L H_2O_2 . In comparison, both seed priming and foliar spray with 5 mmol/L H_2O_2 resulted in the highest filled grain weight/panicle. In addition, filled grain weight/hill was also improved when the plants were treated with H_2O_2 . Seed priming and foliar-sprayed with 5 mmol/L H_2O_2 resulted in a significantly higher filled grain weight/hill than the untreated plant (Figure 1E, F).

Drought stress also caused increases in the unfilled grain weight/panicle and the unfilled grain weight/

hill by 79.49% and 34.23% compared to the untreated plant. Seed priming with H_2O_2 increased plant yield by decreasing the unfilled grain weight/panicle and the unfilled grain weight/hill. Also, foliar-spray with H_2O_2 was even more effective than the seed priming method since the unfilled grain weight/panicle, and the unfilled grain weight/hill were lower than those in the untreated group and the seed priming treatment (Figure 1G, F).

One-thousand-grain weight was significantly reduced by 24.74% when the plant was subjected to drought stress. Seed priming with 1 and 5 mmol/L H_2O_2 , as well as a foliar spray with 5 mmol/L H_2O_2 , slightly improved 1 000-grain weight by 22.63, 19.87, and 17.67%, respectively (Figure 1I).

Pearson's correlation coefficient among the results was presented in Table 3. It was found that plant height was not positively correlated with yield-related traits, while root dry weight (RDW) was positively correlated with shoot dry weight (SDW), the total number of panicles (TP), number of filled grain/hill (NFH), and filled grain weight/hill (FWH). Shoot dry weight was positively correlated with the number of tillers (NT), number of unfilled grains/hill (NUH), and 1 000-grain weight (TWH). For yield-related components, harvest index was positively correlated with panicle length (PL), number of filled grains/hill, filled grain weight/hill, number of filled grains/panicle (NFP), and filled grain weight/panicle (FWP). The number of filled grains/hill was positively correlated with root dry weight, the number of tillers, harvest index, filled grain weight/hill, 1 000-grain weight, and filled grain weight/panicle.

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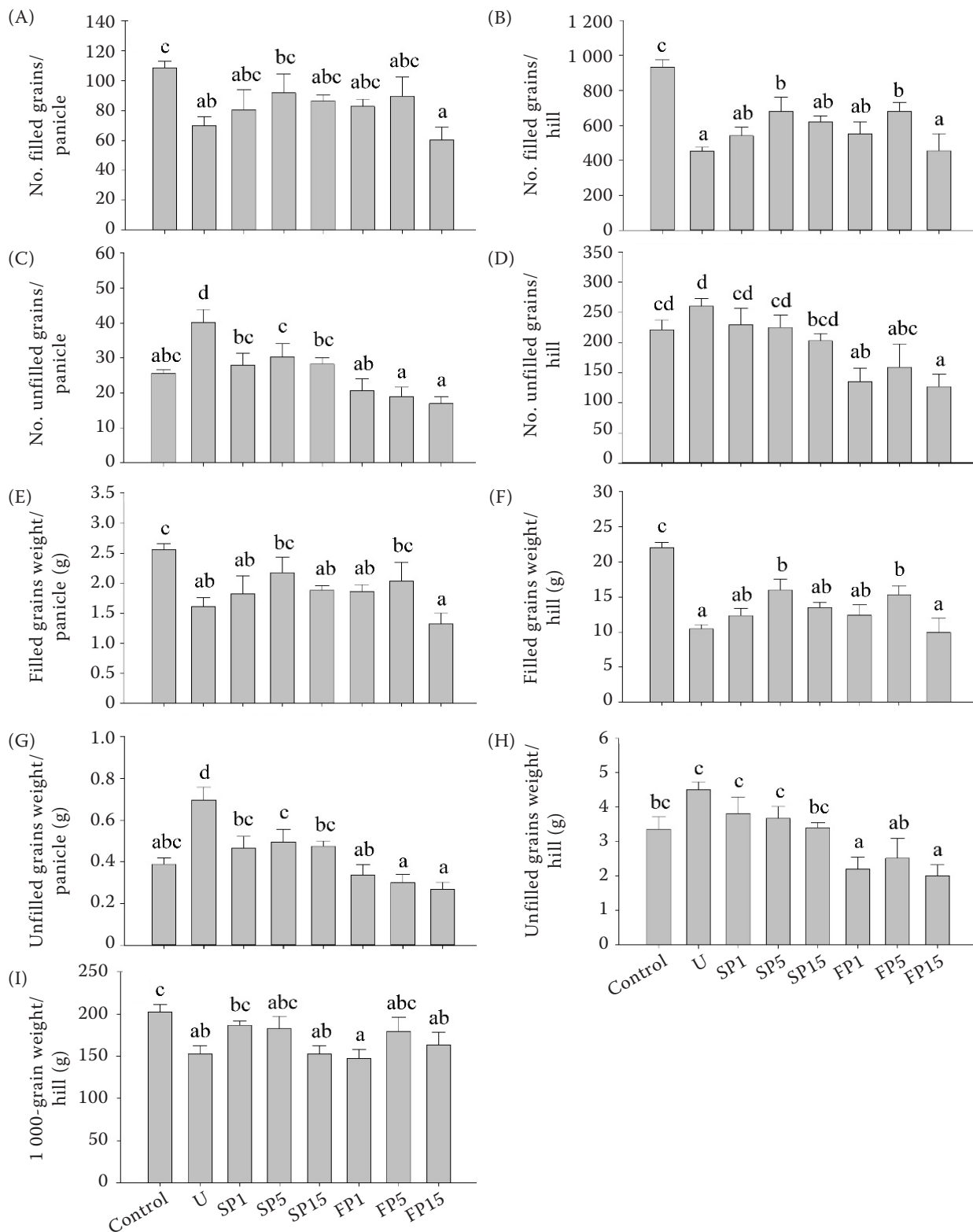


Figure 1. The effects of hydrogen peroxide (H_2O_2) on yield components of rice cv. KDML 105 under drought stress. Data are shown as the mean \pm standard error ($n = 5$). (A) Number of filled grains/panicle; (B) number of filled grains/hill; (C) number of unfilled grains/panicle; (D) number of unfilled grains/hill; (E) filled grain weight/panicle; (F) filled grain weight/hill; (G) unfilled grain weight/panicle; (H) unfilled grain weight/hill, and (I) 1 000-grain weight. U – untreated; SP1 – 1 mmol/L seed priming; SP5 – 5 mmol/L seed priming; SP15 – 15 mmol/L seed priming; FP1 – 1 mmol/L foliar spray; FP5 – 5 mmol/L foliar spray; FP15 – 15 mmol/L foliar spray

Table 3. Pearson's correlation between parameters in drought-stressed rice

	H120	RDW	SDW	NT	TP	HI	PL	NB	NFH	NUH	FWH	UWH	TWH	NFP	NUP	FWP	UWP
H120		-0.310	-0.128	-0.315*	-0.646**	0.258	0.391*	-0.012	0.146	0.203	0.144	0.239	-0.337*	0.376*	0.378*	0.366*	0.391*
RDW			0.481**	0.284	0.446**	0.225	-0.166	-0.137	0.423**	0.075	0.474**	-0.017	0.354*	0.265	-0.061	0.299	-0.139
SDW				0.485**	0.058	-0.134	0.026	0.058	0.194	0.521**	0.220	0.457**	0.506**	-0.029	0.349*	-0.009	0.285
NT					0.405**	0.190	-0.228	-0.336*	0.409**	0.360*	0.386*	0.298	0.935**	-0.074	-0.137	-0.089	-0.170
TP						-0.051	-0.302	-0.206	0.034	-0.265	0.018	-0.304	0.359*	-0.161	-0.513**	-0.179	-0.525**
HI							0.335*	-0.032	0.902**	-0.138	0.902**	-0.201	0.221	0.807**	-0.244	0.812**	-0.286
PL								0.430**	0.250	-0.181	0.277	-0.203	-0.141	0.480**	-0.060	0.502**	-0.086
NB									-0.094	-0.289	-0.105	-0.219	-0.315*	0.114	-0.051	0.109	0.005
NFH										0.043	0.988**	-0.043	0.410**	0.786**	-0.164	0.772**	-0.223
NUH											0.070	0.965**	0.416**	-0.210	0.860**	-0.177	0.813**
FWH												-0.022	0.432**	0.783**	-0.125	0.795**	-0.194
UWH													0.356*	-0.260	0.858**	-0.233	0.874**
TWH														-0.056	-0.057	-0.019	-0.092
NFP															-0.171	0.984**	-0.209
NUP																-0.130	0.970**
FWP																	-0.176
UWP																	

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed). H120 – plant height at 120 days; RDW – root dry weight; SDW – shoot dry weight; NT – no. of tillers; TP – total no. panicles; HI – harvest index; PL – panicle length; NB – no. of the primary branch; NFH – no. filled grains/hill; NUH – no. unfilled grains/hill; FWH – filled grain weight/hill; UWH – unfilled weight/hill; TWH – 1 000 grain weight/hill; NFP – no. filled grains/panicle; NUP – no. unfilled grains/panicle; FWP – filled grains weight/panicle; UWP – unfilled grains weight/panicle

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DISCUSSIONS

In plants, H_2O_2 plays diverse roles in various biochemical and physiological processes. Due to its long life span and versatility, H_2O_2 can traverse through cellular membranes and potentially act as a signaling molecule in the stress signal transduction pathway. These pathways can then trigger various responses involved in stress acclimation. This study showed that seed priming with appropriate concentrations of H_2O_2 , i.e., 1 mmol/L and 5 mmol/L, enhanced plant growth under non-stressed as well as drought conditions. In cannabis, using H_2O_2 as a priming agent showed an increase in dry weight under normal conditions (Golizadeh et al. 2015). Also, seed priming with H_2O_2 shows higher dry weight and root length under drought conditions in wheat (Hameed and Iqbal 2014).

It was proposed that seed priming could leave the "stress memory," which, in turn, activates the stress response mechanisms after germination (Chen and Arora 2013). Seed priming with a high concentration of H_2O_2 , however, resulted in the reduction of root and shoot biomass. Despite acting as a signaling molecule, a high concentration of H_2O_2 may lead to oxidative stress that causes cellular damage and results in programmed cell death (Gupta et al. 2016).

Yield components, including tiller number, harvest index, panicle length, as well as number and weight of filled grains, were also improved by H_2O_2 priming. Priming with 5 mmol/L H_2O_2 was the most effective for improving rice yield. It has been hypothesised that seed priming could activate stress-responsive systems such as antioxidant activation, hormonal regulation, and abscisic acid signaling (Chen and Arora 2013, Lutts et al. 2016). Cheng et al. (2017) reported that the genes associated with stress were observed at the early imbibition stage. Besides, genes related to protein synthesis, carbohydrate metabolism, and signaling were up-regulated at the late imbibition stage. Marthandan et al. (2020) proposed that the seed priming method can induce physiological adaptation, which in turn increases crop yield under drought stress. In the review, the stress memory left in the seed can faster, and stronger activate the gene and transcription after post-germination. In wheat, the yield was also enhanced under drought stress when primed with an appropriate concentration of H_2O_2 (Hameed and Iqbal 2014). Habib et al. (2020) reported that H_2O_2 priming has the potential to increase tiller, 1 000-grain weight, and grain yield/plant. Optimal concentrations of H_2O_2 can enhance abiotic stress

tolerance through the modulation of multiple physiological processes, such as photosynthesis, stomatal movement, osmotic adjustment, and ROS detoxification (Quan et al. 2008, Niu and Liao 2016, Sayed and Gadallah 2019). In mustard, seed priming with H_2O_2 showed significant increases in antioxidant enzyme activities (Hossain and Fujita 2013). This result indicates that H_2O_2 could trigger the ROS detoxification mechanism, which is crucial for maintaining the structural and membrane integrity of cellular organelles and keeping them fully functional in plants under abiotic stresses (Hossain et al. 2015). The study in *Caklie martima* Scop. showed that seed priming with 120 μ mol H_2O_2 improved malondialdehyde content (MDA), lipid peroxidation index in both drought and salt stress (Ellouzi et al. 2017). A recent study in rice also found that seed priming with 5 mmol/L H_2O_2 decreased electrolyte leakage but increased relative water content (Jira-anunkul and Pattanagul 2020).

Although foliar-spray with H_2O_2 did not affect shoot growth and biomass during drought stress, it is interesting that spraying with H_2O_2 did increase root growth in which may increase the plant's capability to access water. Yield components, on the other hand, were significantly improved by foliar application with H_2O_2 . The number of filled grain-filled grain weight and harvest index were higher than the untreated plant, especially in 5 mmol/L treatment. Rice reproductive stage at the flowering stage is highly susceptible to drought leading to pollen and spikelet sterility, which causes a significant reduction in yield (Yang et al. 2019). There was evidence suggested that H_2O_2 application helps increase yield in many plants. Orabi et al. (2018) reported that spraying with 2 mmol H_2O_2 induced the antioxidant enzyme activities, e.g., catalase, ascorbate peroxidase, while decreased lipid peroxidation in the canola plant. The onion yield was significantly improved when applied with 1–2 mmol/L H_2O_2 (Semida 2016). Therefore, it is suggested that physiological changes caused by H_2O_2 help the plants maintain growth under unfavourable conditions, which results in a better yield.

In addition, it is noteworthy that foliar-spray with H_2O_2 significantly lower the unfilled grain number and weight, which was more prominent than the seed priming method. A previous study suggested that H_2O_2 might activate the defense system as well as improve water status in rice under drought stress. It is also reported that H_2O_2 treatment decreased the endogenous H_2O_2 , which resulted in lower malondialdehyde content. H_2O_2 accumulation also triggers

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kinase protein and targets calcium homeostasis, ion channel, phosphatase, transcription factor, and ABA signaling response to stress (Černý et al. 2018). The previous evidence suggested that pretreatment with H₂O₂ increased abscisic acid and osmolytes concentration in maize leaves (Terzi et al. 2014). Abscisic acid is well-documented for triggering stomatal closure and activating stress-responsive gene expression (Ali et al. 2020). The antioxidant system, in particular, is triggered by H₂O₂ signaling and may account for the observed increase in total yield as well as preventing yield loss. Taken all together, it is suggested that H₂O₂ application prevents the plant from ROS attack and maintains normal physiological processes, therefore, prevent yield loss (Sohag et al. 2020). Similar to the seed priming treatment, a high concentration of H₂O₂ does not enhance yield production but instead results in yield loss.

In conclusion, both seed priming and foliar spray with H₂O₂ contributed to improving rice cv. KDML 105 growth and agronomic traits under drought stress. Using 5 mmol H₂O₂ as a priming agent or foliar application was the most effective concentration as indicated by increases in filled grain weight, filled grain number, and harvest index. Similar to the seed priming method, foliar application with H₂O₂ provided benefits in increasing yield production and also preventing yield loss. Therefore, the decision on each technique depends on available facilities, cost, and farmer's preference.

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