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Effects of Short- and Long-Term Temperature on Seed Germination, Oxidative Stress and Membrane Stability of Three Rice Cultivars (Dular, KDML105 and Riceberry)

Borriboon, W.[1], Lontom, W.[1], Pongdontri, P.[2], Theerakulpisut, P.[3] and Dongsansuk, A.[4]*

[1]*Department of Biology, Faculty of Science, Khon kaen University, Khon kaen, Thailand*
[2]*Department of Biochemistry, Faculty of Science, Khon kaen University, Khon kaen, Thailand*
[3]*Salt-tolerant Rice Research Group, Khon Kaen University, Khon kaen, Thailand*
[4]*Department of Plant Science and Agricultural resources, Faculty of Agriculture, Khon kaen University, Khon kaen, Thailand*

ABSTRACT

The reduction in rice productivity as a result of elevated daily temperature due to climate change is a major concern for Thailand. This study aimed to investigate hydrogen peroxide and malondialdehyde (MDA) content and electrolyte leakage of rice seedlings grown from seeds exposed to different temperature (25°C, 35°C and 40°C) treatments over a short (one week) and long (two weeks) period before germination. Three rice cultivars were investigated, Dular, KDML105 and Riceberry. The experiment was designed in RCBD with six replications. The results indicated that Riceberry seeds produced a greater percentage of normal seedlings after both short- and long-term heat treatment (40°C). By contrast, KDML105 seeds exposed to 40°C for one and two weeks gave rise to the highest percentage of abnormal seedlings. The highest oxidative stress indicated by the accumulation of hydrogen peroxide was found in abnormal seedlings of cvs. KDML105 and Riceberry after short- and long-term heat (40°C) exposure, respectively. The effect of heat stress on membrane stability was indicated by MDA content and electrolyte leakage. MDA content was the highest in abnormal seedlings of cv. Riceberry after heat exposure for two weeks. High electrolyte leakage due to both short- and long-term high temperature treatment was found in abnormal seedlings of all the rice cultivars. Heat exposure to rice seeds at 40°C for one week induced the highest percentage of abnormal seedlings in KDML 105 coinciding with the highest hydrogen

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E-mail addresses:
wantida@kkumail.com (Borriboon, W.),
watalo@kku.ac.th (Lontom, W.),
paweena@kku.ac.th (Pongdontri, P.),
piythe@kku.ac.th (Theerakulpisut, P.),
Danoma@kku.ac.th (Dongsansuk, A.)
* Corresponding author

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peroxide content and membrane damage. These results provide crucial information for consideration in breeding programmes for heat-tolerant rice cultivars.

Keywords: Temperature stress, seed germination, oxidative stress, membrane stability

INTRODUCTION

Rice (*Oryza sativa*) is one of the most important crops in Thailand. The main rice production area is found in Northeastern Thailand. One of the most common rice cultivating methods in this region is the sowing of seeds before the rainy season, a practice that often exposes seeds to high temperatures. The current maximum temperature of the warmest month in Thailand and Northeastern Thailand are approximately 41°C and 40°C, respectively (Thai Meteorological Department, 2017). In addition, the maximum temperature of the warmest month in Thailand is predicted to rise by approximately 1.5-2°C (Trisuart et al., 2011). Peng et al. (2004) reported that rice becomes heat sensitive and this reduces grain yield by 10% during the reproductive stage for every 1°C rise in daily temperature, i.e. every increase by 1°C leads to a reduction of 10 % yield. High temperatures also affect seed germination and the physiology of seedlings. Essemine et al. (2010) reported that ungerminated seeds and damaged embryo were found in wheat seeds exposed to 45°C. Piramila et al. (2012) found that *Vigna mungo* seeds showed a significantly decreased germination percentage and seed vigour index after heat exposure. Similarly, the germination percentage of *Cassia tora* seeds exposed to 40°C, 50°C and 60°C for 10 days decreased by 85%, 63% and 32%, respectively compared to seeds incubated at room temperature (Pant et al., 2012).

High temperatures lead to oxidative burst, indicated by the production of reactive oxygen species (ROS), namely, superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•) (Hammond-Kosack & Jones, 1996). These ROS exert a toxic effect in cells via lipid peroxidation, protein degradation, DNA damage, plasma membrane damage (Fridovich, 1986; Halliwell & Gutteridge, 1989), electrolyte leakage (Abass & Rajashekar, 1991), cell structure damage (Mittler et al., 2004) and other physiological changes such as declining photosynthetic rate, increasing respiration rate (Paulsen, 1994) and finally, cell death (Maxwell et al., 1999). The autocatalytic peroxidation of membrane lipids and pigments and modification of membrane permeability and function are the main effects of ROS (Hasanuzzaman et al., 2012). Lipid peroxidation is indicated by malondialdehyde (MDA) content. A high temperature of 40/45°C (day/night temperature) leads to MDA content increases in rice and maize by 1.8-fold and 1.2- to 1.3-fold, respectively. Similarly, the H_2O_2 levels at 40/35°C showed a 1.9- to 2.0-fold elevation in rice and 1.4- to 1.6-fold elevation in maize relative to their control (Kumar et al., 2012). Membrane stability indicated by electrolyte leakage was used in one study for screening thermal tolerance in wheat (Saadalla et al., 1990), and MDA content was also found to be an indicator of membrane stability (Fan et al., 2015).

In Thailand, Khao Dawk Mali 105 (KDML105) is a Thai rice variety commonly known as jasmine rice. KDML105 is well-known in the global market because of its distinctive characteristic of a white colour, long grains and slender shape. When it is cooked, it is soft and releases a natural aromatic fragrance (Ranna et al., 2016; Bureau of Rice Research and Development, 2017). Riceberry is the most famous Thai brown rice. It is a dark violet grain and contains a high amount of antioxidants. It is soft and releases an aroma after cooking (Rice Science Center and Rice Gene Discovery, 2017). Finally, Dular is a traditional rice variety from India (Wang et al., 1998) and is classified as highly heat tolerance using as donor parents in a breeding programme (Magnibas et al., 2014). This research focused on determining the effects of seed pretreatment with different temperatures on seed germination, oxidative stress and membrane stability in seedlings of these three rice cultivars i.e. Dular (Magnibas et al., 2014), KDML 105 and Riceberry, all of which have different levels of heat tolerance.

MATERIALS AND METHOD

Seed Germination and Stress Conditions

Dry seeds of three rice cultivars, that is, Dular (obtained by Biotechnology Research and Development Office, Thailand), KDML105 and Riceberry (obtained by Assist. Prof. Dr. Jirawat Sanitchon, KKU), were used as the experimental materials. This experiment was carried out in the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand during the period April-August 2015. For heat treatment, 20 sterilised seeds were placed in a Petri dish each and incubated at 25°C (optimum temperature for germinating seeds and growing worldwide plants), 35°C (optimum temperature for growing rice plants) and 40°C (maximum temperature of the warmest month in Northeastern Thailand) for one week (minimum duration for which dry rice seeds can be exposed to high temperatures under dry ungerminated seed broadcasting cultivation) and two weeks (moderate duration for which dry rice seeds can be exposed to high temperatures under dry ungerminated seed broadcasting cultivation). After heat exposure, the seeds were placed on germination paper and put in a plastic box under humidity control by spraying with water. The germination boxes were placed in a growth chamber (25°C and 80% of RH) under normal light. Seeds were considered as germinated after the radicles had emerged to a length of 2 mm. Germination was recorded at 14 days according to AOSA (1990) and then germination percentage (% GP) was calculated according to ISTA (1985) (Equation 1) and Pirasteh-Anosheh et al. (2011). Normal and abnormal seedlings were collected for measurement of hydrogen peroxide and malondialdehyde content and electrolyte leakage.

$$\text{Germination percentage (\% GP)} = \frac{\text{No. of normal seedlings x 100}}{\text{No. of total seeds}} \quad (1)$$

Measurement of Hydrogen Peroxide Content

Hydrogen peroxide content was determined according to Velikova et al. (2000). Sample tissues (0.5 g) were ground in an ice bath with 5 ml of 0.1% w/v trichloroacetic acid (TCA) and centrifuged at 12,000×g for 15 min. The supernatant (0.5 ml) was transferred to a 15-ml test tube and 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide were added to the supernatant and mixed throughly. The mixture was measured spectrophotometrically at 390 nm.

Measurement of MDA Content

The assay of lipid peroxidation measured the amount of MDA formation according to Heath and Packer (1968). Sample tissues (0.1 g) were transferred to a 15-ml test tube to which 1.4 ml of distilled water was added; this was then mixed using a vortex mixer. Thiobarbituric acid (TBA) reagent (1.5 ml of 0.5% (w/v) TBA in 20% (w/v) TCA) was then added and mixed using a vortex mixer. The mixture was boiled in a water bath for 25 min. The reaction was stopped by placing the tube on ice for 5 min and then centrifuged at 1000×g for 10 min to remove cell debris. The absorbance of the supernatant was measured spectrophotometrically at 532 nm and 600 nm.

Measurement of Electrolyte Leakage

Electrolyte leakage (EL) measurement (in %) was determined according to Bajji et al. (2001). Fresh seedling samples (0.1 g) were placed in a 15-ml test tube containing 10 ml of deionised water and incubated at room temperature for 24 h. The electrical conductivity (EC_1) of the suspension was measured, and the tube was then heated to 100°C for 15 min. The samples were then cooled to 25°C and the final electrical conductivity (EC_2) was measured.

Experimental Designs and Statistical Analysis

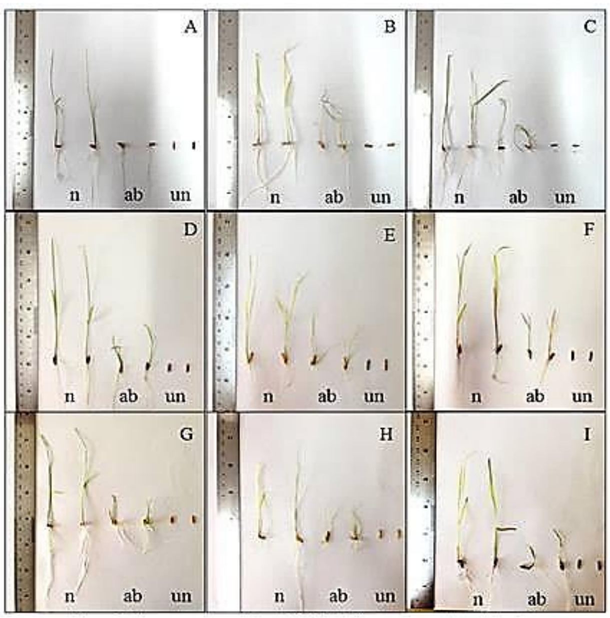
The experiment was carried out in a randomised completely block design (RCBD) with six replications. Data were analysed using analysis of variance (ANOVA) at the significant level of p=0.05 and means comparison or means separation among various treatments was determined through Duncan's multiple range tests (DMRT) at p=0.05 using the Statistical Package for the Social Sciences software (SPSS) for windows version 17.0.

RESULTS AND DISCUSSION

Effects of High Temperatures on Characteristics of Seed Germination and Seed Germination Percentage

Characteristics of seed germination in three rice varieties, Dular, KDML105 and

Riceberry are shown in Figure 1. Normal seedlings (n) in all rice varieties showed white and long roots. Root length ranged from 3 to 6 cm. The leaf blade was green and approximately 5-6 cm long. Leaf numbers per plant was approximately 2-3. Abnormal seedlings (ab) showed white roots and their root length was 0.5-3 cm. The leaf blade was yellow or brown, folded and curved and approximately 3-5 cm long. The leaf number per plant was in the range of 1-2. In ungerminated seeds (un), the seed coats were dark brown and black. A milky liquid was expressed when the seeds were crushed. The ungerminated seeds were classified as dead seeds according to ISTA (1985).



Dular KDML 105 Riceberry

Figure 1. The characteristics of normal seedlings (n), abnormal seedlings (ab) and ungerminated seeds (un) of three rice cultivars: Dular (A, D and G), KDML105 (B, E and H) and Riceberry (C, F and I) grown from seeds treated at 25°C, 35°C and 40 C for one week (left seedlings) and two weeks (right seedlings)

Many physiological changes under high temperature stress may be reflected in the abnormality of seed germination. The interaction between the cultivars and temperature (cv x Temp) and between the cultivars and exposure time (cv x Time) was significant for all instances of seed germination and the interaction of cv x Temp x Time was also significant (Table 1). The significant differences were mainly due to high temperature adversely affecting seed germination of all the rice cultivars (Figure 2 and Table 1). Riceberry seeds produced the highest percentage of normal seedlings after the seeds were exposed to 40°C for one week (62.5 ± 1.44%). However, when the seeds were treated at 40°C for two weeks, the Dular seeds showed the highest percentage of normal seedlings (61.67 ± 1.67%). The exposure temperature of 40°C for one and two weeks (36 ± 6% and 27.5 ±1.44%) induced the lowest percentage of normal seedlings in cv. KDML105. This result was supported by the work of Abernethy (1989), who reported reduction of seed germination percentage in rice due to high temperature (51°C) exposure, and Ali et al. (2013), who reported that the high temperature of 42 ± 3°C resulted in slow seed germination and decreased the percentage of seed germination. Moreover, Akman (2009) showed that temperature exposure at 35, 38 and 41°C reduced the germination of rice and sorghum. The results of abnormal seedlings are shown in Figure 2C and D. The results show that KDML105 was the highest percentage of abnormal seedlings after temperature exposure at 40°C for one week (70 ± 2.88%) and two weeks (70%) compared to other rice cultivars. Spears et al. (1997) found that high temperature at 38/33°C (day/night) resulted in a low percentage of normal seedlings but exhibited a higher percentage of abnormal seedlings. This study found that Dular seeds were the most tolerant to high temperature, showing the lowest percentage of abnormal seedlings after the seeds were exposed to 40°C for one and two weeks (20 ± 2.8% and 18.33 ± 1.67%). No instances of temperature treatment affected the percentage of ungerminated seeds in all the rice cultivars.

Table 1
Analysis of variance of the effects of rice seed cultivars, temperatures and temperature-exposure duration on the percentage of normal seedling germination, abnormal seedling germination and ungerminated seeds under Thailand experimental conditions

Sources	% Normal Seedlings	% Abnormal Seedlings	% Ungerminated Seeds
		Mean square	
Cultivars (cv)	1766.90**	3680.57**	844.51**
Temperature (Temp)	323.35**	36.96	29.79
Temperature-Exposure Duration (Time)	1622.51**	1340.01**	8.96
cv * Temp	545.57**	1004.06**	117.29**
cv * Time	440.35**	471.13**	37.85**
Temp * Time	1043.97**	810.29**	8.68
cv * Temp * Time	241.57**	212.65**	68.40**
Error	35.70	35.07	10.70

** Indicates significant at $p \leq 0.05$ probability levels

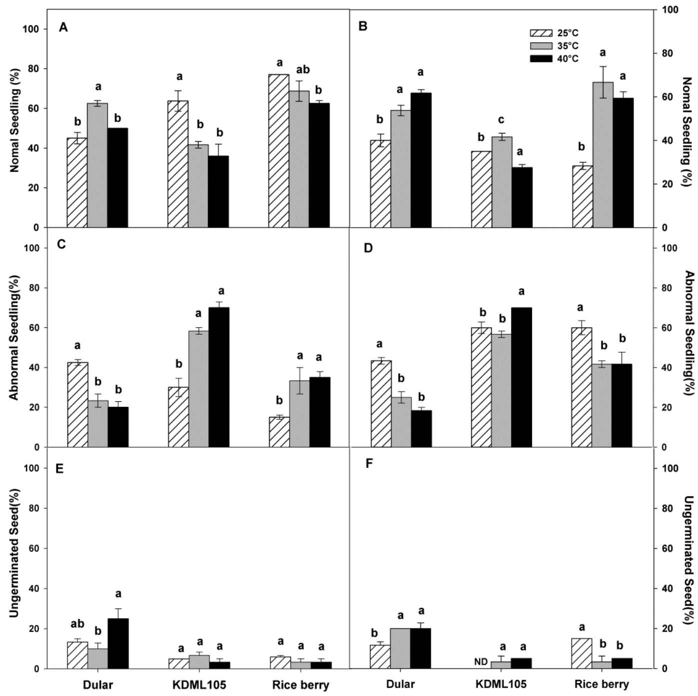


Figure 2. Effect of exposing seeds to different temperatures (25°C, 35°C and 40°C) for one (A, C, E) and two (B, D, F) weeks before germination on percentages of normal seedlings (A and B), abnormal seedlings (C and D) and ungerminated seeds (E and F) in rice cvs. Dular, KDML105 and Riceberry. The values are means ± SE (n=3 to 4). ND=not determined. Different letters in each cultivar indicate significant differences among temperature treatments tested by DMRT at p≤0.05

Effect of High Temperature on Oxidative Stress and Membrane Stability of Rice Seeds

Hydrogen peroxide accumulation in the abnormal seedlings grown from seeds treated at 40°C for one week was higher (0.5-3-fold) than in those treated at 35°C for one week. The increasing trend of hydrogen peroxide accumulation was found in the abnormal seedlings germinated from Dular and KDML105 seeds treated at 35 and 40°C for two weeks (Figure 3B). This result related to the interaction of cv x Time, cv x Seedling x Temp and cv x Time x Seedling x Temp, which showed significant differences (Table 2). From this study, the increasing content of hydrogen peroxide in the abnormal seedlings grown from seeds treated at 35 and 40°C suggested that abnormal seedlings suffered from oxidative stress induced by ROS, leading to growth inhibition, similar to the results reported by Schöffl et al. (1999). The reactive oxygen species can oxidise membrane lipid resulting in cell membrane damage (Bowler et al., 1992), leading to cell death (Abernethy et al., 1989). Ali et al. (2013) reported that at the germination stage, rice showed high H_2O_2 content due to heat exposure. Timabud (2015) also indicated that rice var. IR64 showed high

H_2O_2 content in high temperature treatment. Normal and abnormal seedlings of rice cv. Riceberry grown from seeds treated at 25°C for two weeks showed the highest level of hydrogen peroxide accumulation (2- and 3-fold higher than seedlings of rice cvs. Dular and KDML105, respectively).

Table 2
Analysis of variance of the effects of rice cultivars, temperatures, temperature-exposure duration and seedling normal status on H_2O_2, MDA and EL of rice seedlings under Thailand experimental conditions

Sources	H_2O_2 mmol/gFW	MDA mmol/gFW	EL (%)
	Mean square		
Cultivars (cv)	5.30 E-8**	0.001**	1376.69**
Temperature-Exposure Duration (Time)	1.05 E-8**	4.90 E-5	26.98**
Temperature (Temp)	5.51 E-8**	0.001**	4644.58**
Seedling Normal Status (Seedling)	1.12 E-7**	0.002**	11078.41**
Seedling * Temp	0.20 E-8**	0.001**	772.50**
cv * Time	1.53 E-8**	0.001**	73.71**
cv * Seedling * Temp	0.27 E-8**	0.007**	51.39**
Time * Seedling * Temp	0.13 E-8**	5.74 E-5^{ns}	107.26**
cv * Time * Seedling * Temp	0.37 E-8**	0.45 E-5^{ns}	78.95**
Error	1.80E-10	2.15E-5	6.46

ns and ** Indicates non-significant and significant at $p \leq 0.05$ probability levels, respectively

The decline in membrane stability can be determined by MDA content and electrolyte leakage (EL) (Fan et al., 2015). A trend of higher MDA content in all the rice cultivars treated with different temperatures for one and two weeks was found in abnormal seedlings rather than in normal seedlings as shown in Figure 3C-D and Table 2 (the interaction of cv x Time and cv x Seedling x Temp was significant for MDA content). The highest MDA content was found in abnormal seedlings of Riceberry (0.13 ± 0.008 µmol/g FW) compared with other rice cultivars (Table 2); MDA content was significant in the different cultivars. Furthermore, EL in the abnormal seedlings grown from seeds treated at 25 and 40°C for one and two weeks was higher than in those grown from seeds treated at 35°C. In addition, the EL percentage in normal seedlings germinated from all the seeds of the rice cultivars treated at 25°C and 40°C for two weeks showed a higher trend than for temperature exposure at 35°C. Ali et al. (2013) reported that MDA content and EL in rice seedlings treated at 42°C for 72 h was higher than for seedlings treated at 42°C for 24 and 48 h. Moreover, Zhang et al. (2005) suggested that heat stress severely affected mesophyll cell damage and induced increased membrane permeability. Either denaturation of proteins or an increase in unsaturated fatty acids caused the higher fluidity in the lipid bilayer of biological membranes. The lipids were then destroyed by the lipid peroxidation process, which produces MDA as a final product (Savchenko et al., 2002). The integrity and functions of biological membranes are sensitive to high temperature; for example, heat stress alters the tertiary and quaternary structures of membrane proteins. Such alterations

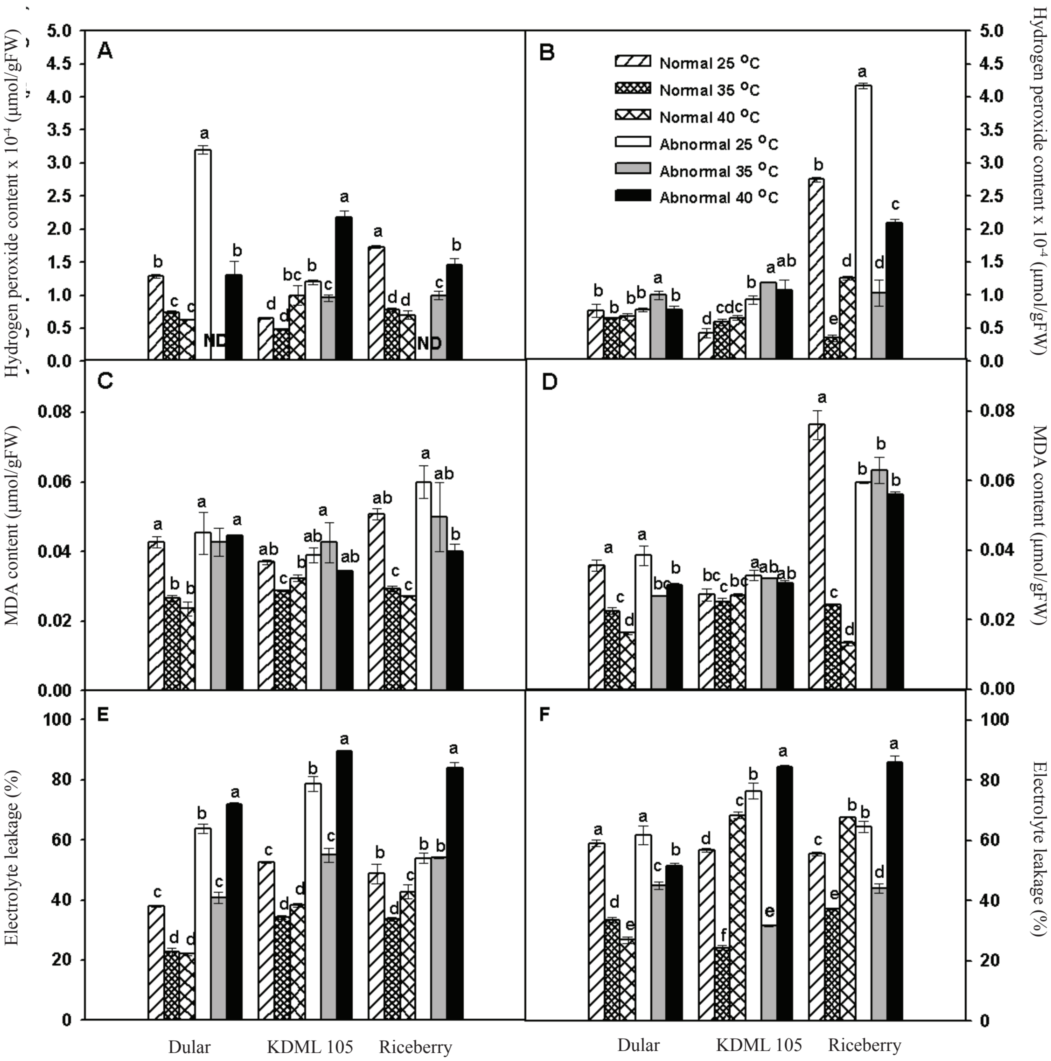


Figure 3. Effect of exposing seeds to different temperatures (25°C, 35°C and 40°C) for one and two weeks before germination on hydrogen peroxide content (A and B), MDA content (C and D) and electrolyte leakage (E and F) in seedlings of rice cvs. Dular, KDML105 and Riceberry. The values are means ± SE (n=3 to 4). ND=not determined. Different letters in each cultivar indicate significant differences among temperature treatments tested by DMRT at p≤0.05

enhance the permeability of membranes (Savchenko et al., 2002), as is evident from the greater loss of electrolytes after heat exposure (Figure 3E-F and Table 2). Even though Riceberry exhibited high oxidative stress and membrane stability after long high temperature exposure in this study, it may have been recovered by ROS scavenging or membrane repair immediately after attack with ROS. Consequently, ROS was unaffected by heat temperature and ROS levels were higher in the normal seedlings.

CONCLUSIONS

From the results, it was concluded that the dry rice seeds exposed to a high temperature (40°C) resulted in a higher percentage of abnormal seedlings, particularly in KDML105. A higher potential for heat tolerance was found in seeds of rice cvs. Dular and Riceberry. Short-term heat exposure (40°C) induced an increasing trend of oxidation stress in all the abnormal seedlings. Seeds of rice cvs. Dular and Riceberry were susceptible to both short- and long-term exposure to low temperature at 25°C, as shown by the increased oxidative stress and decreased membrane stability. Both short- and long-term heat exposure at 40°C induced increased membrane damage in the abnormal seedlings, indicated by the high EL percentage.

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