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Research Article

Screening Rice Lines Tolerant to Low Temperature at the Germination Stage Using Thermogradient bar

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Abstract

Background and Objective: Breeding for superior rice varieties for upland drylands requires parent lines that tolerate low temperatures. Thermogradient bar can be used to screen plant tolerance to temperature during the germination stage. This study aimed to evaluate several tolerance variables to low-temperature stress during the germination stage and select hybrid rice lines tolerant to low temperatures using a Thermogradient bar device. **Materials and Methods:** The experiment was conducted at the Biology Research Center Laboratory, Indonesian Institute of Sciences (LIPI), Cibinong, Bogor. It used a Complete Randomized Block Design with five replications. Rice tolerance to low temperatures during the generative phase was tested in the highlands in the KP. Tongkoh, Karo (1430 m above sea level and 19°C temperature). A total of 10 promising lines and five control varieties were used in the test. The control varieties used were Ciherang, Stubagendit, Mekongga as sensitive comparative varieties and Sigambiri Merah and Sigambiri Putih as tolerant comparative varieties. Thermogradient bar was used for testing tolerance to low temperatures at several temperature levels, namely 13, 16, 18 and 28°C. **Results:** The rice genotypes tested had better responses than the sensitive comparative varieties. However, the responses differed from the comparative tolerant Sigambiri under low-temperature stress conditions. The Karo 121 DHP line is a sensitive genotype at <18°C, and the Karo 122 DHP line is the most tolerant. **Conclusion:** Rice selection using Thermogradient bar can be a rapid test method for low-temperature stresses in the germination phase.

Key words: Thermogradient bar, germination, upland rice, low-temperature, sensitive

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Low temperature affects the rice plants at all stages of growth. It can cause severe seedling injury and male sterility resulting in severe yield losses[1]. According to another study[2], symptoms of rice crop damage due to low temperatures are a late and small percentage of germination. In the vegetative phase, leaves turn yellow, plant postures are shorter and tillers are reduced.

Some local highland rice varieties are tolerant of low temperatures, such as Sigambiri Putih, Sigambiri Merah, Mayas, Selasih[3,4]. The weaknesses of these local varieties are long life and low yield. For this reason, a national rice breeding program needs to utilize local varieties as donors of genes tolerant to low-temperature stress to set up highland rice varieties.

The tolerance of rice plants to low temperatures is an important character, which needs particular attention in plant breeding in the upland. In implementing this variety, screening for tolerance to low temperatures is quite tricky and requires a long duration. Besides, it also requires expensive facilities and many breeding cycles to study plants' agronomic, physiological and qualities[5].

So far, the selection for low-temperature characters has focused on the flowering phase. Therefore, the rice panicle formation stage is a critical phase of low-temperature stress. However, it is crucial to make a breakthrough for selection at an earlier growth stage, such as the seed stage. This stage of selecting will reduce the area of land required to select rice plants compared to selecting plants after flowering. Only plants that can germinate and grow well in low-temperature conditions are then selected to determine the age of flowering and productivity.

According to another study[6], germination is critical for rice with low temperatures. Screening in this phase has expected to reduce genetic material to speed up the assembly process. Fast and accurate screening techniques for tolerance toward low temperatures are essential breeding stages. One of them is simulating low-temperature stress conditions in the laboratory using a thermogradient device. Thermogradient bar is a tool that can screen from 2-45°C[7]. Therefore, this tool is used to select lines at a specific temperature range quickly and effectively by looking at germinating to a specific age limit.

This study aimed to determine the response of rice seed germination at various temperature levels using thermogradient. Furthermore, it revealed the relationship with rice tolerance at the generative phase in the highland field.

MATERIALS AND METHODS

Genetic material: A total of 15 rice genotypes has used in this study. It consisted of 10 advanced generations of upland rice lines selected in the Tanah Karo highlands of North Sumatra. Then, two local upland rice varieties, namely Sigambiri Merah and Sigambiri Putih and three popular superior varieties that are sensitive to the low-temperature, namely Situ Bagendit, Ciherang and Mekongga in Table 1.

Low-temperature tolerance test in the seedling phase: The test has conducted at the Biology Research Center Laboratory, Indonesian Institute of Sciences (LIPI), Cibinong, Bogor. The study took place in April-June 2017, using a Thermogradient bar device assembled by LIPI. This low-temperature screening[8]. An indicator to see tolerance to low temperatures used is the percentage of growing ability at temperatures below 18°C.

The test was carried out using five replications. A total of 5 individuals were planted for each test to match the size of each temperature column in the Thermogradient device (10×15 cm). Replications are distinguished based on the seedling period. Observations were made for 9 days after seedling. The temperature gradient used was adjusted to the temperature that occurs on the device based on the separation of rooms-a total of 10 rooms were used for growth testing. The temperature gradient formed is T_1:31°C, T_2:30°C, T_3:28°C, T_4:27°C, T_5:24°C, T_6:22°C, T_7:20°C, T_8:18°C, T_9:16°C, T_{10}:13°C.

Observations included optimum germination temperature, the critical temperature for low-temperature stress, germination power, number of leaves, shoot length, root length, number of normal sprouts, fresh weight and dry weight. Data were analyzed with variance using SAS v.9 and Microsoft Office Excel 2019.

Table 1: Rice genotypes for tolerance tests towards low temperatures

Genotype	Explanation
DHP. KARO 103	Inpago LIPIGO 1/Sigambiri Merah
DHP. KARO 106	Gapai/Padi Mandailing
DHP. KARO 108	Gapai/Padi Mandailing
DHP. KARO 114	Luhur 2
DHP. KARO 119	
DHP. KARO 120	
DHP. KARO 121	
DHP. KARO 122	
DHP. KARO 124	
DHP. KARO 125	
Stubagendit	Sensitive test
Sigambiri Merah	Tolerance test
Ciherang	Sensitive test
Sigambiri Putih	Tolerance test
Mekongga	Sensitive test

Data from this study, screening rice

The sensitivity index measured germination, leaves, shoot length, root length, number of normal sprouts, fresh weight and dry weight. The low-temperature stress sensitivity index (S) has been calculated using the equation, as follows[9]:

$$S = \frac{\frac{1-Yp}{Y}}{\frac{1-Xp}{X}}$$

Where:

S = Low-temperature stress sensitivity index

Yp = Average value of a genotype that gets low-temperature stress

Y = Average value of a genotype that does not get a low-temperature stress

Xp = Average of all genotypes receiving low-temperature stress

X = Average of all genotypes that do not get low-temperature stress

The criteria for determining the tolerance level to low temperatures are the value of S <0.5 categories of tolerant genotype, 0.5 <S <1.0 category of tolerant medium genotype and S> 1.0 for sensitive genotype.

Low-temperature tolerance tests in the research field: Field testing has been conducted to validate the rice tolerance response results at low temperatures using this Thermogradient bar. The test was carried out from July, 2017 to March, 2018 at the Berastagi Experimental Garden, Tongkoh, Karo District, at an altitude of 1340 m above sea level and an average temperature of 19°C. Twenty plants were planted in the field of each genotype. Each line was planted in a plot with a 1×5 m spacing of 25×25 cm. Fertilization used was 300 kg ha^{-1} NPK+100 kg ha^{-1} Urea. Maintenance is carried out optimally. Weeding is carried out twice, before supplementary fertilization I and II. Observations included: Plant height, number of tillers, 50% flowering age, number of panicles, total chlorophyll at 4 and 12 WAP (the week after planting), percentage of fertile pollen and fresh yield/panicle.

RESULTS AND DISCUSSION

Performance of rice genotype response in germination phase towards various temperature levels using Thermogradient bar: Rice is a crop that presents sensitivity to cold, especially in the germination phase, which leads to high economic losses[10]. In the initial stages, rice genotypes were tested at ten temperature levels (T_1-T_{10}) to see the response pattern in the germination phase. The test results showed there were variations in responses in all genotypes. Generally, plant growth decreases along with the lower temperature. The distribution pattern of the growth of 15 genotypes of rice seeds at various temperature levels showed a similar trend. Sigambiri Putih showed the highest results in all temperature ranges based on the genotype germination power percentage. Decreased germination power in most genotypes occurred at T_6 (temperature 22°C) and T_{10} (temperature 13°C) and the mortality rate was very high. At the same time, the two local varieties of Sigambiri Merah and Sigambiri Putih have high germination power despite being at a low temperature (13°C).

Seeds one called to have high growth power if they have a germination value of 80%. Based on this, the optimum temperature for rice seed germination occurs at 22-28°C, while stress at low temperatures starts to appear at 18°C. Findings agree with the previous study[11], higher temperature conditions during the final stages of rice seed development (seed filling and maturation) are known to cause damage to both rice yield and rice kernel quality.

At 31°C, seed germination was standard for all genotypes. The average percentage of growth at this temperature was 85%. The average growth percentage then decreased to 22.13% at 13°C. According to another study[12], the temperature range of 25-30°C is ideal for germination and seedling's initial growth, but the seeds continue to germinate under a temperature elevation of 35°C. Different temperature ranges cause changes in several characteristics besides the average number of normal sprouts, including the length of time to germinate and the vigour of sprouts. At low temperatures, rice seeds generally become slow to germinate, whereas at higher temperatures germinated seeds often are unable to develop shoots. Not infrequently the roots that have grown die because of drought.

Table 1 showed that DHP Karo 122 was the most tolerant line in the vegetative phase compared to other lines with an average value of growing power at cold temperatures (13, 16 and 18°C) of 44%. However, this line was still lower in growth than sensitive tests (Stubagendit, Mekongga and Ciherang). Stubagendit growth power at temperatures <18°C was 63% while Sigambiri Putih was 95%. The most intolerant low-temperature genotype was the DHP KARO 121 line with growing power of 24%.

At low temperatures (13°C), Sigambiri Putih (tolerant test) has a percentage of leaves growing by 56%. In contrast, Sigambiri Merah (tolerant test) and Ciherang (sensitive test) to the upland had a percentage of the number of leaves growing equivalent at temperatures at 13°C, which was equally 12%.

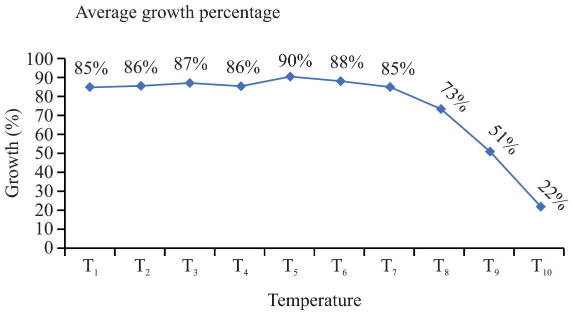

Fig. 1: Average rice germination power at different temperatures

(T_1 = 31°C, T_2 = 30°C, T_3 = 28°C, T_4 = 27°C, T_5 = 24°C, T_6 = 22°C, T_7 = 20°C, T_8 = 18°C, T_9 = 16°C and T_{10} = 13°C)

Analysis of various effects of low temperature on rice genotypes: Based on the data of rice germination power at different temperatures in Fig. 1, it revealed that at 28°C the average percentage of plant growth was stable, so for further analysis, 28°C temperature is used as a controlled temperature against other low temperatures. It is in line with the previous research[6], which stated that a temperature of 28°C provides ideal germination conditions for all seeds. For low-temperature stresses 18, 16 and 13°C have been used.

From Table 2, it can be seen that temperature and genotype have a significant effect on root length of 9.78, shoot length of 9.69, percentage of normal sprouts of 6106.29 and plant fresh weight of 0.02, but no significant effect on dry weight character 0.01. The interaction of temperature with genotype does not significantly affect all observed characters.

When compared between the three low temperatures in Table 3, in general rice at 18°C is higher than 13 and 16°C. It showed that the lower the temperature, the more stressed the plants. The temperature value of 13°C was root length 0.40, shoot length 1.19, normal germination percentage 6.4, fresh weight 0.16 and dry weight 0.11. while for temperature, 18°C root length was 2.69, shoot length was 2.07, normal germination percentage was 54.6, fresh weight was 019 and dry weight was 0.15. Temperatures below the optimum level of the plant caused a decrease in the speed of growth and metabolic processes. As a result, the time needed to complete the plant's life cycle will be longer, as the temperature drops to a critical point, the plant suffered damage until death. Low-temperature stress can negatively affect rice plants during the germination phase, the vegetative growth phase and the reproductive phase[2]. The impact of low temperature on rice growth is seedling growth which causes discolouration of leaves (yellowing of leaves). Other impacts were a small number of tillers, slow flowering time, abnormal panicle exertion, increases panicle sterility, irregular panicle maturation and decreases yield[13].

Low-temperature stress had no significant effect on plant dry weight but significantly affected root length, shoot length, percentage of normal sprouts and fresh plant weight. Sigambiri Merah showed values of root length of 4.15, shoot length of 4.29, percentage of normal germination of 72.00, fresh weight of 0.27 and dry weight of 0.14. Furthermore, Sigambiri Putih showed values of root length of 4.14, shoot length of 4.050, percentage of normal germination of 81.00, fresh weight of 0.28 and dry weight of 0.14 higher than other genotypes in Table 4.

It showed that Sigambiri Merah and Sigambiri Putih tolerate low-temperature stress compared to the sensitive Stubagendit, Ciherang and Mekongga varieties.

Sigambiri Merah at a temperature of 13°C had a root length value of 1.01, shoot length of 0.98, normal germination percentage of 1.29, fresh weight of 0.75 and dry weight of 0.28. Furthermore, Sigambiri Putih at a temperature of 13°C, the root length value was 0.95, the shoot length was 0.94, the normal germination percentage was 0.59, the fresh weight was 0.66 and the dry weight was -0.23. From Table 5, it is revealed that, in general, the genotypes tested have lower values than the sensitive comparative varieties (Stubagendit, Mekongga and Ciherang).

Index of rice plant sensitivity towards low-temperature stress: The plant can adapt to defoliation by allocating more to tolerance and less to growth and defense[14]. Plants that experience stress generally experienced a decrease in yield. The rate of yield reduction for each plant genotype varied depending on the adaptability of each genotype to stress conditions. The tolerance level of a rice genotype in a low-temperature stress environment can be determined based on the stress index value, which is calculated based on the comparison of results under stress conditions to yield under optimum conditions.

Lower temperature stress on rice germination resulted in decreased all observed variables compared to the optimum temperature. Based on the sensitivity index of low-temperature stress on the variable percentage of germination, showed that the temperature treatment revealed the diversity of rice genotype tolerance to low-temperature stress (Table 5).

In general, the tolerant comparative varieties, Sigambiri Merah and Sigambiri Putih had a lower sensitivity index value than the sensitive comparative varieties, namely Ciherang, Mekongga and Stubagendit on all germination variables. The rice lines used showed different sensitivity index values for each germination variable observed.

Table 2: Square of the five characters evaluated

Diversity sources	Root length	Shoot length	Normal sprouts (%)	Fresh weight	Dry weight
Temperature	525.89	970.21**	81946.22**	0.48**	0.03
Deuteronomy (temperature)	7.86**	2.82**	796	0.003	0.01
Genotype	9.78**	9.69**	6106.29**	0.02**	0.01
Temperature × genotype	1.17	2.34	836.69	0.002	0.01
Error	0.81	0.75	467.43	0.0008	0.006

**Significantly different at 1% test level

Table 3: Root length, shoot length, percentage of normal sprouts, average fresh and dry weight of rice under low-temperature stress

Temperature treatments	Root length	Shoot length	Normal sprouts (%)	Fresh weight	Dry weight
28°C (control)	6.41^{a}	8.03^{a}	84^{a}	0.33^{a}	0.15^{b}
18°C	2.69^{b}	2.07^{b}	54.6^{b}	0.19^{b}	0.15^{a}
16°C	1.29^{c}	0.76^{c}	31.8^{c}	0.16^{c}	0.12^{b}
13°C	0.40^{d}	0.19^{d}	6.4^{d}	0.16^{c}	0.11^{b}

Numbers followed by different letters in each parameter showed a significant difference (5%) according to DMRT: Duncan multiple range test

Table 4: Growth of 15 rice genotypes with low-temperature treatment (≤18°C)

Genotypes	Root length	Shoot length	Normal sprouts (%)	Fresh weight	Dry weight
DHP. KARO 103	2.35^{def}	2.42^{cde}	34.00^{ef}	0.18^{d}	0.10^{b}
DHP. KARO 106	2.06^{ef}	2.06^{e}	34.00^{ef}	0.18^{d}	0.09^{b}
DHP. KARO 108	2.12^{ef}	2.21^{de}	33.00^{ef}	0.19c^{d}	0.09^{b}
DHP. KARO 114	1.885^{f}	2.24^{de}	33.00^{ef}	0.18^{d}	0.09^{b}
DHP. KARO 119	2.29^{def}	2.090^{e}	27.00^{f}	0.18^{d}	0.11^{b}
DHP. KARO 120	2.38d^{ef}	2.49^{cde}	29.00^{ef}	0.17^{d}	0.18^{a}
DHP. KARO 121	2.55^{bcde}	2.62^{cde}	27.000^{f}	0.21^{bc}	0.09^{b}
DHP. KARO 122	2.77^{bcd}	2.79^{bcd}	44.00^{de}	0.22^{bc}	0.09^{b}
DHP. KARO 124	2.50^{cdef}	2.26^{de}	39.00^{ef}	0.19^{d}	0.09^{b}
DHP. KARO 125	2.08^{ef}	2.38^{cde}	33.00^{ef}	0.21b^{c}	0.16^{ab}
Stubagendit	3.04^{bc}	2.93^{bc}	57.00^{cd}	0.21^{b}	0.12^{ab}
Sigambiri Merah[b]	4.15^{a}	4.29^{a}	72.00^{ab}	0.27^{a}	0.14^{ab}
Ciherang[a]	3.03^{bc}	3.35^{b}	55.00^{cd}	0.22^{b}	0.12^{b}
Sigambiri Putih[b]	4.14^{a}	4.050^{a}	81.00^{a}	0.28^{a}	0.14^{ab}
Mekongga[a]	3.17^{b}	3.29^{b}	65.00^{bc}	0.23^{b}	0.13^{ab}

[a]Sensitive comparison variety, [b]Tolerant comparison varieties, the number followed by different letters in each parameter showed a significant difference (5%) according to DMRT

Table 5: Low-temperature stress sensitivity index of 15 rice genotypes with low-temperature stress treatment at the thermogradient bar germination stage

	Temperature 18°C					Temperature 16°C					Temperature 13°C				
Genotypes	Root length	Shoot length	Normal sprouts (%)	Fresh weight	Dry weight	Root length	Shoot length	Normal sprouts (%)	Fresh weight	Dry weight	Root length	Shoot length	Normal sprouts (%)	Fresh weight	Dry weight
DHP. KARO 103	0.59	1.14	0.61	0.52	-0.26	0.82	1.01	1.15	0.59	-0.24	1.10	1.02	1.35	0.57	-0.41
DHP. KARO 106	0.77	1.16	0.74	0.66	-0.06	0.94	1.05	1.01	0.68	-0.07	1.10	1.02	1.35	0.66	-0.21
DHP. KARO 108	0.82	1.12	0.82	0.77	-0.23	0.95	1.02	0.75	0.79	-0.28	1.06	1.02	1.35	0.81	-0.31
DHP. KARO 114	0.70	0.98	0.45	0.59	-13.61	1.02	0.97	1.12	0.61	-0.27	1.10	1.02	1.35	0.68	-0.21
DHP. KARO 119	0.73	1.02	0.71	0.36	-0.12	0.94	1.03	1.27	0.55	-0.15	1.05	0.99	1.27	0.53	-0.18
DHP. KARO 120	0.82	1.10	0.60	0.68	-5.95	1.01	1.05	1.27	0.79	-0.20	1.07	1.01	1.35	0.79	-0.28
DHP. KARO 121	0.59	0.89	0.45	0.70	-0.43	0.95	1.08	1.17	0.81	-0.43	1.10	1.02	1.35	0.62	-0.51
DHP. KARO 122	0.54	0.97	0.70	0.69	-0.47	0.90	1.00	1.08	0.75	-0.47	1.00	0.99	1.24	0.83	-0.43
DHP. KARO 124	0.72	1.04	0.50	0.58	-0.34	0.79	0.93	0.85	0.57	-0.34	0.99	1.01	1.28	0.71	-0.41
DHP. KARO 125	0.67	1.15	0.67	0.65	-3.29	0.97	1.05	1.21	0.78	-0.28	1.02	1.00	1.28	0.79	-0.34
Stubagendit	0.72	1.11	0.38	0.55	-0.51	0.88	1.00	0.59	0.56	-0.45	1.07	1.02	1.35	0.65	-0.41
Sigambiri Merah	0.48	0.91	0.05	0.43	0.27	0.74	0.97	0.16	0.68	0.31	1.01	0.98	1.29	0.75	0.28
Ciherang	0.60	0.95	0.43	0.62	-0.46	0.85	0.97	0.86	0.69	-0.43	0.97	0.97	1.13	0.70	-0.46
Sigambiri Putih	0.53	0.78	0.16	0.41	-0.01	0.77	0.97	0.27	0.61	-0.16	0.95	0.94	0.59	0.66	-0.23
Mekongga	0.45	0.81	0.11	0.44	0.09	0.80	0.92	0.43	0.63	0.09	0.98	1.00	1.35	0.68	0.04

Validation of rice plant tolerance at low-temperature stress in the field: The average temperature of the environment in the experimental field is 19°C. Based on the daily temperature data presented in Fig. 1, the highest temperature is 25.2°C and the lowest is 15.2°C. The trial field has an altitude of 1340 m above sea level. So, the temperature is quite low. The previous

Table 6: Average character values of 15 genotypes at low-temperature stress in Karo

Genotypes	Plant height (cm)	Number of tillers max (clump stems^{-1})	Number of panicles (items)	Score of SPAD 4 MST	Score of SPAD 12 MST	Flowering age (days)	Fertile pollen (%)	Yield/ panicle (g)
DHP. KARO 103	Death	Death	Death	Death	Death	Death	Death	Death
DHP. KARO 106	88.36±5.55	16.6±6.44	0	20.24±6.59	35.84±2.89	154.4±4.25	3.00±5.4	0
DHP. KARO 108	76±8.82	19.25±8.54	11±10.82	26.45±2.82	39.03±4.18	151.33±2.31	27.38±31.22	0.28±0.24
DHP. KARO 114	77.19±10.19	20.55±5.57	4.5±3.54	24.76±11.02	38.09±3.70	152±2.19	0.55±0.79	0.19±0.19
DHP. KARO 119	77.67±2.51	36.67±0.57	15±10.54	26.1±6.10	41.73±1.53	151.33±2.31	15.94±15.87	1.74±1.49
DHP. KARO 120	77.67±2.52	36.67±5.03	15±10.54	26.1±6.10	41.73±1.53	151.33±2.31	15.94±15.87	1.74±1.49
DHP. KARO 121	73.36±4.42	25.1±7.08	0.2±0.63	26.28±6.26	39.6±3.56	150.4±1.26	11.40±20.46	0.1±0.14
DHP. KARO 122	78.08±14.26	29.8±12.93	39.67±55.82	24.28±12.05	40.28±4.54	153.25±4.27	1.73±2.11	8.05±12.99
DHP. KARO 124	78.98±6.11	28±11.59	7.75±7.85	24.9±6.25	35.9±6.05	150±0	9.13±9.13	0.49±0.29
DHP. KARO 125	70.83±2.02	29.67±14.36	0	26.3±1.21	39.2±3.64	150±0	3.01±2.77	0
Stubagendit	62.02±4.86	35.3±12.53	0	30.07±5.07	36.95±6.77	143.85±6.39	2.57±4.04	0
Sigambiri Merah	117.45±10.55	24.3±9.35	28.55±30.06	28.43±9.77	51.65±5.97	140.45±5.31	45.32±23.84	10.99±2.42
Ciherang	56.62±6.44	22.67±9.39	0	24.13±8.64	38.08±4.02	157±3.61	0	0
Sigambiri Putih	130.74±10.05	27.47±10.44	85.65±21.64	29.99±6.62	47.35±5.30	150.71±5.36	73.22±20.90	14.42±6.86
Mekongga	49.69±3.73	29.14±11.41	0	23.31±4.39	38.71±2.06	158±2.34	1.09±1.54	0

study results[15] showed that yield loss from rotten seedlings caused by low temperatures is a serious and global problem in rice production.

The results of the field trials indicate the DHP genotype. KARO 125 had the shortest plant height, averaging 70.83±2.02 cm. This plant's height is shorter than the low temperature tolerant comparative plants (Sigambiri Merah and Sigambiri Putih) and higher than the sensitive comparative plants (Stubagendit, Ciherang and Mekongga). The highest genotype was DHP. KARO 106 (88.36 cm), while the other genotypes had plant heights ranging from 70.83-78.98 cm in Table 6. Plant height is one of the selection criteria for rice plants, but high growth does not guarantee production and tolerance to low temperatures.

The genotypes DHP.KARO 119 and DHP.KARO 120 owns the maximum number of tillers (37 tillers). While the DHP. KARO 106 (16 tillers) genotype owns the minimum number of tillers compared to all genotypes.

The highest yield is for the DHP. KARO 122 (8.05±12.99 g) genotype. This yield value is higher than the sensitive comparative variety (0 g) and lower than the tolerant comparative variety. The lowest yield was found in the DHP. KARO 106 genotype and 125 (0 g) meanwhile for the DHP. KARO 103 genotype, no plant can survive in low-temperature conditions.

At field testing, it appears that sensitive comparative varieties (Stubagendit, Ciherang and Mekongga) were unable to produce seeds so production is zero. It is due to low-temperature stress during the generative/reproductive phase causing structural and functional abnormalities in the reproductive organs, failure of fertilization or early abortion of seeds or fruit, resulting in decreased yields[16].

The promising lines were also capable of producing in low-temperature stress conditions, except for DHP Karo 103 and 125, however, their yield is still lower compared to tolerant comparative varieties (Sigambiri Merah and Putih). The DHP Karo 122 line is a line that has a higher yield compared to other lines with a higher number of filled seeds. It is also in line with results from testing using the Thermogradient bar, where the percentage of normal sprouts was 44%, which is the highest compared to the percentage of normal sprouts in other lines.

This information showed that the selection of rice plants using Thermogradient bar can be used as a rapid test method for low-temperature stresses that occur in the germination phase. With this method, it is expected that selection could be made more effectively and efficiently, both in terms of time and cost.

The main findings of this study are, that the DHP Karo 121 strain was a sensitive genotype at <18°C and significantly different from the white Sigambiri resistant check. The DHP Karo 122 line was the most tolerant of the vegetative phase compared to the other lines, with an average growth rate of 44% at cold temperatures (13, 16 and 18°C). From the research in the field, the genotype of rice tolerant in low-temperature areas is DHP.

Seeds one called to have high growth power if they have a germination value of 80%. Based on this, the optimum temperature for rice seed germination occurs at 22-28°C, while stress at low temperatures starts to appear at 18°C. The results of this study confirmed the research of other study[17], which showed that a decrease in germination occurs at temperatures below 13°C. In his research, it was known that most temperature-sensitive genotypes only have germination power values below 35%. Therefore, the selection of rice seeds for the tolerant character at low temperature is carried out at 18°C.

At 31°C, seed germination was standard for all genotypes. The average percentage of growth at this temperature was 85%. The average growth percentage then decreased to 22.13% at 13°C. According to another study[18], the optimal temperature range needed by rice plants for seed germination and initial seedling growth is from 25-35°C. Different temperature ranges cause changes in several characteristics besides the average number of normal sprouts, including the length of germination and the vigour of sprouts. At low temperatures, rice seeds generally become slow to germinate, whereas germinated seeds are often unable to develop shoots at higher temperatures. Not infrequently, the roots that have grown die because of drought.

Based on the data on rice germination power at different temperatures (Fig. 1), it was revealed that at 28°C, the average percentage of plant growth was stable, so for further analysis, 28°C temperature is used as a controlled temperature against other low temperatures. It is in line with the research of other work[6], which stated that a temperature of 28°C provides ideal germination conditions for all seeds. 18, 16 and 13°C have been used for low-temperature stresses.

When compared between the three low temperatures in Table 3, rice at 18°C is higher than 13 and 16°C. It showed that the lower the temperature, the more stressed the plants. Temperatures below the optimum level of the plant caused a decrease in the speed of growth and metabolic processes. As a result, the time needed to complete the plant's life cycle will be longer, as the temperature drops to a critical point, the plant suffered damage until death. Low-temperature stress can negatively affect rice plants during the germination phase, the vegetative growth phase and the reproductive phase[2]. The impact of low temperature on rice growth is seedling growth which causes discolouration of leaves (yellowing of leaves). Other impacts were a small number of tillers, slow flowering time, abnormal panicle exertion, increases panicle sterility, irregular panicle maturation and decreased yield[13].

Plant tolerance is the ability to survive and produce in stress conditions or environments[19]. Plants that experience stress generally experienced a decrease in yield. The rate of yield reduction for each plant genotype varied depending on the adaptability of each genotype to stress conditions. The tolerance level of a rice genotype in a low-temperature stress environment can be determined based on the stress index value, which is calculated based on the comparison of results under stress conditions to yield under optimum conditions.

Estimating rice genotype tolerance using stress sensitivity index values calculated based on certain variables showed different values. It made it difficult to determine the sensitivity index of germination variables used to classify rice tolerance to low-temperature stress. The selection of variables to determine the tolerance of a genotype to low-temperature stress should be made comprehensively because determining the tolerance level based on only one variable resulted in an invalid assessment[20].

Then, a visual observation also showed that both the Sigambiri Merah and Sigambiri Putih varieties have similar performance on the same temperature gradient. Therefore, it is suspected that the low-temperature stress critical point in the Ciherang variety occurs in the generative phase only. Therefore, further testing is required for the generative phase at the target location. The ideal target location is above 900 m above sea level, with an average ambient temperature of <18°C.

CONCLUSION

Low-temperature stresses begin to appear at 18°C. The Karo 121 DHP line is a sensitive genotype at <18°C and markedly different from the Sigambiri Putih. The Karo 122 DHP line is the most tolerant in the vegetative phase compared to other lines, with an average value of growing power at cold temperatures (13, 16 and 18°C) of 44%. Field research showed that tolerant rice genotypes in low-temperature areas are DHP. KARO 122 yielded 8.05±12.99 g/panicle, which exceeded the sensitive comparison varieties. Rice selection using Thermogradient bar can be a rapid test method for low-temperature stresses in the germination phase.

SIGNIFICANCE STATEMENT

This is the first study conducted reporting that local white and red Sigambiri rice are rice plants that are tolerant of low temperatures. This can be screened with the help of a Thermogradient bar tool in the germination phase and proven in the field, namely KP. Tongkoh, Karo, Indonesia (1430 m above sea level and 19°C temperature). The DHP Karo 122 genotype was found to be the most tolerant in the vegetative phase with an average growth value at cold temperatures (13, 16 and 18°C) of 44% and in line with the results of field research.

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