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Seed Quality Selection of Several Genotypes of Brown Rice (*Oryza sativa* L.) under High-Temperature Stress

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Abstract

Climate change impacts increasing temperature, and environmental factors can affect the capacity and rate of seed germination. This study aimed to determine and study the effect of temperature changes on the viability and vigor of brown rice seeds and the morphology of the sprouts produced. The variation of temperature used was 28-40^{0}C, and ten genotypes of brown rice seeds used were Pulen Mudiak, Pulen Kandih, Pulen Marapak, Pulen Talao, 64, Sibandung, Silalang, Timbo Abu, Labuah Baru, and Melayu. The results showed that every 1^{0}C increase in temperature affects rice germination percentage. The optimum temperature for the germination of ten genotypes of brown rice tested was a temperature range of 28-33^{0}C, with a germination value of >80%, the maximum critical temperature 37^{0}C and at 38-40 ^{0}C no brown rice seeds germinated. Pulen Marapak has the highest maximum growth potential of 90,3% at 28^{0}C and 10% at 37^{0}C. The increase in temperature also damages brown rice roots and shoots of ten genotypes, with the average root length being 6,7-10,1 cm and shoot length being 8-11,5 cm at 28^{0}C.

Keywords: antioxidant, brown rice, germination, seed quality, tolerant

1. Introduction

Comparison of air temperature in a particular year with the average period (1981-2010), better known as the air temperature anomaly. Overall, Indonesia in 2016 was the hottest year with an anomaly value of 0,8°C, while 2020 was the second hottest year with an anomaly value of 0,7°C. Compared with the 2019 anomaly value of 0,6°C, there is an increase in temperature anomaly every year by 0,1°C. The difference in the average temperature in December 2020 with November 2020 from the 86 Meteorological Station observation stations in Indonesia generally shows a negative value, with the most significant difference being -2,5°C at the Gewayantana-East Flores Meteorological Station, while the most significant positive difference found in the Rahadi Oesman, Ketapang Meteorological Station at 0,6°C (BMKG, 2021).

Temperature changes that occur significantly affect the growth and development of plants in Indonesia, especially food crops like rice, the staple food in Indonesia. Rice seed comprises a maternal caryopsis coat, a diploid embryo, and a triploid endosperm, in which most of the nutrients are stored in the form of starch, protein, lipids, and other trace substances (Qiu et al. 2015). Rice is rich in genetic diversity, one of that, namely brown rice that has high nutritional and antioxidant content.

Rice has a critical temperature for the germination stage, 16-19°C (low), 45°C (high) and the optimum at 18-40°C while for the optimum growth and development stage of sprouts at 25-30°C with a maximum critical at 35°C (Yoshida, 1978). Temperature increases more than optimum can disrupt metabolism, protein aggregation, enzyme denaturation, and damage to cells and plant cell organelles (Taiz and Zeiger, 2006). According to Soepandi (2014), the initial effect of high-temperature stress is a more fluid lipid bilayer on the plasma membrane and then cell death.

The adaptability of plants to high-temperature stress varies between genotypes. Tenorio et al. (2013), rice plants tend to tolerate high temperatures in the vegetative but are sensitive in the generative. Therefore, by testing the quality of seeds, it is possible to determine the characteristics of each genotype. This research is also the first step to assemble a new high-yield variety tolerant to high temperatures as reported by Jaisyurahman et al. (2019) that planting high-yielding varieties tolerant to high-temperature stress is the only way to prevent a decline in rice productivity due to the impact of climate change. However, the temperature increase will seriously impact agricultural production.

Based to Anne (2014), brown rice contains carbohydrates (85%), fiber (7%), B vitamins (thiamin, riboflavin, vitamin B-6, folate, and niacin), magnesium, phosphorus, calcium, and potassium. The local brown rice used in this study is the result of exploration in Pasaman and West Pasaman Regencies, where there are ten genotypes of brown rice found with a fluffy rice texture; Pulen Mudiak, Pulen Kandih, Pulen Marapak, Pulen Talao, 64, Sibandung, Silalang, Timbo Abu, Labuah Baru, Melayu (Madya, 2020).

2. Materials and Methods

This research was carried out from October 2020 to February 2021 at the Seed Technology Laboratory, Faculty of Agriculture, Andalas University. The brown rice genotypes tested were Pulen Mudiak, Pulen Kandih, Pulen Marapak, Pulen Talao, 64, Sibandung, Silalang, Timbo Abu, Labuah Baru, and Melayu using the Top of Paper method with petri dish. Seeds were germinated in the Growth Chamber at 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C, 37°C, 38°C, 39°C, 40°C and 80% of RH under normal light. Each treatment was tested for 50 seeds with three replications.
The observed variables:

First Count Test (%)

First Count Test (FCT) is calculated based on the percentage of regular sprouts on the first count, five days afetr germinated;

$$\text{FCT} = \frac{\sum Seed\ germinate\ normally\ count\ I}{\sum All\ seed\ germinated} \times 100\%$$

Germination (%)

Germination was recorded at 5 days and 14 days according to ISTA (2004), calculated with the following formula:

$$\text{G} = \frac{\sum Seeds\ that\ germinate\ normally\ \ I+II}{\sum All\ seeds\ germinated} \times 100\%$$

Maximum Growth Potential (%)

Normal and abnormal seedlings were collected at 14 days, calculated by:

$$\text{MGP} = \frac{\sum Seed\ that\ germinate\ normally + abnormally}{\sum All\ seeds\ germinated} \times 100\ \%$$

Root and Shoot Growth Test (cm)

The physiological seed quality approach is carried out by observing the growth of sprouts, such as root and shoot growth (Root and Shoot Growth Test).

3. Results and Discussion

The nature of rice tolerance to high-temperature stress can be improved through breeding programs such as selection. Each stage of plant growth certainly has a different effect. The germination stage is easily controlled by observing seed quality through seed viability and vigor. The viability and vigor of the seeds accumulated were higher, the plant response at the next stage will also be good.

First Count Test (%)

First Count Test is one of the indicators to determine seed vigor where average sprout growth is observed five days after germination. The first count test of ten varieties of brown rice tested shown in Table 1.

Table 1. First Count Test of Brown Rice at 5 Days After Germination

Genotypes	Temperature (^{0}C)												
	28	29	30	31	32	33	34	35	36	37	38	39	40
------ (%) -----													
Pulen Mudiak	83,6	79,0	76,6	78,0	76,6	78,0	79,0	71,6	41,0	4,3	0,0	0,0	0,0
Pulen Kandih	86,6	83,6	84,6	79,6	77,3	74,6	75	64,3	24,7	5,6	0,0	0,0	0,0
Pulen Marapak	83,3	76,3	77,0	79,0	78,3	76,0	74,6	54,6	20,3	6,3	0,0	0,0	0,0
Pulen Talao	87,3	82,6	85,6	81,3	82,6	82,3	74,6	56,0	40,6	3,6	0,0	0,0	0,0
64	86,3	83,7	84,6	76,6	80,3	79,3	77,3	58,6	41,0	4,6	0,0	0,0	0,0
Sibandung	87,6	87,3	87,0	81,0	77,3	79,0	79,0	58,6	38,0	5,0	0,0	0,0	0,0
Silalang	86.0	87,0	84,6	81,3	78,3	80,3	78,3	58,3	50,3	6,3	0,0	0,0	0,0
Timbo Abu	83,0	85,6	86,3	84,6	82,3	75,3	76,3	47,3	27,3	1,6	0,0	0,0	0,0
Labuah Baru	88,3	83,3	85,0	81,7	79,3	76,0	74,6	55,0	40,0	12,3	0,0	0,0	0,0
Melayu	75,3	82,3	87,3	81,6	78,6	68,6	72,3	51,0	29,0	4,6	0,0	0,0	0,0

Seed germination is a critical process ensuring the continuity of life in plants that depend on it as the exclusive mode of propagation (Bewley, 1997). Abiotic factors such as high temperature halt seed germination slow or delay germination by affecting the expression or regulation of alpha-amylase (Njeri *et al.,* 2019). The effect of temperature on germination varies among plant species and cultivars. Some varieties experienced a decrease in the First Count Test percentage with increasing temperature, such as Sibandung and some varieties varied in response to each temperature increase. The ten rice genotypes tested had a high percentage of First Count Test (>80) at 28-33^{0}C. Labuah baru has the highest First Count Test of all the genotypes tested at 37°C was 12.3%.

Germination and Maximum Growth Potential (%)

Germination and Maximum Growth Potential of seeds can detect the level of viability. Figure 1 explains the curve of the percentage of germination seed.

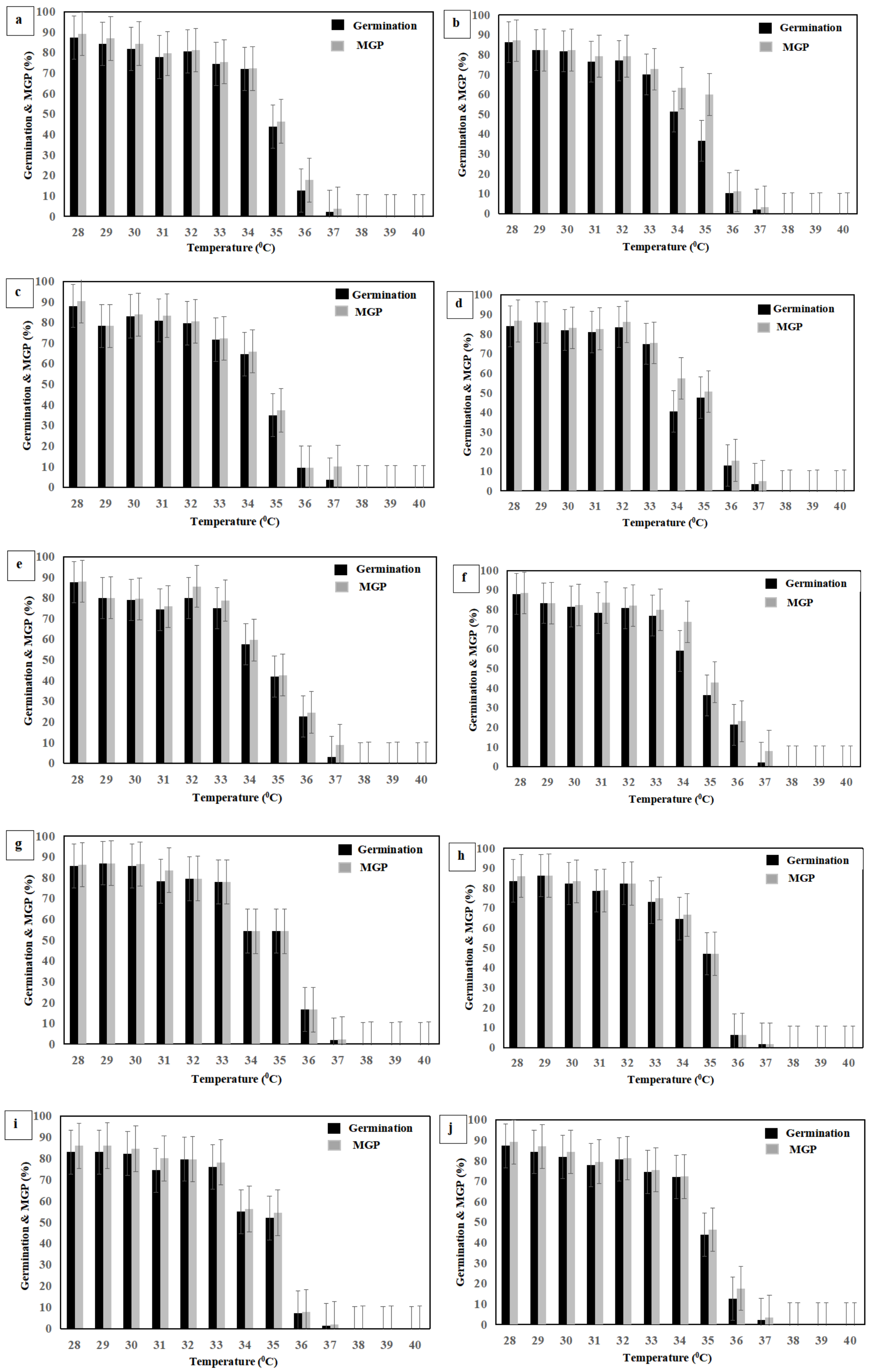


Figure 1. Germination and Maximum Growth Potential (%) curve for seeds (a) Pulen Mudiak (b) Pulen Kandih (c) Pulen Marapak (d) Pulen Talao (e) 64 (f) Sibandung (g) Silalang (h) Timbo Abu (i) Labuah Baru (j) Melayu

Brown rice germinated at 28-370C, with every genotype having different responses. Pulen Marapak could germinate at 370C with a low maximum growth potential of only 10%. This result showed the optimum temperature for ≥80% germination at 28-330C. High temperature inhibited seed germination by decreasing metabolic activity such as enzyme denaturation. In germinating rice seeds, a-amylase was synthesized in the embryo and aleurone layer and converted endosperm starch into metabolizable sugars to be energy seeds to germinate and nourish the young seedling (Hakata et al., 2012). Sari et al. (2021) stated that the denaturation of the enzyme alpha-amylase in the varieties of Anak Daro, Batang Piaman, Cisokan, and Inpari 30 occurred in a temperature range of 40-450C. Bewley and Black (1984) also reported that germination is usually retarded at 400C and the activities of enzymes involved in the degradation of stored components are also depressed at 400C.

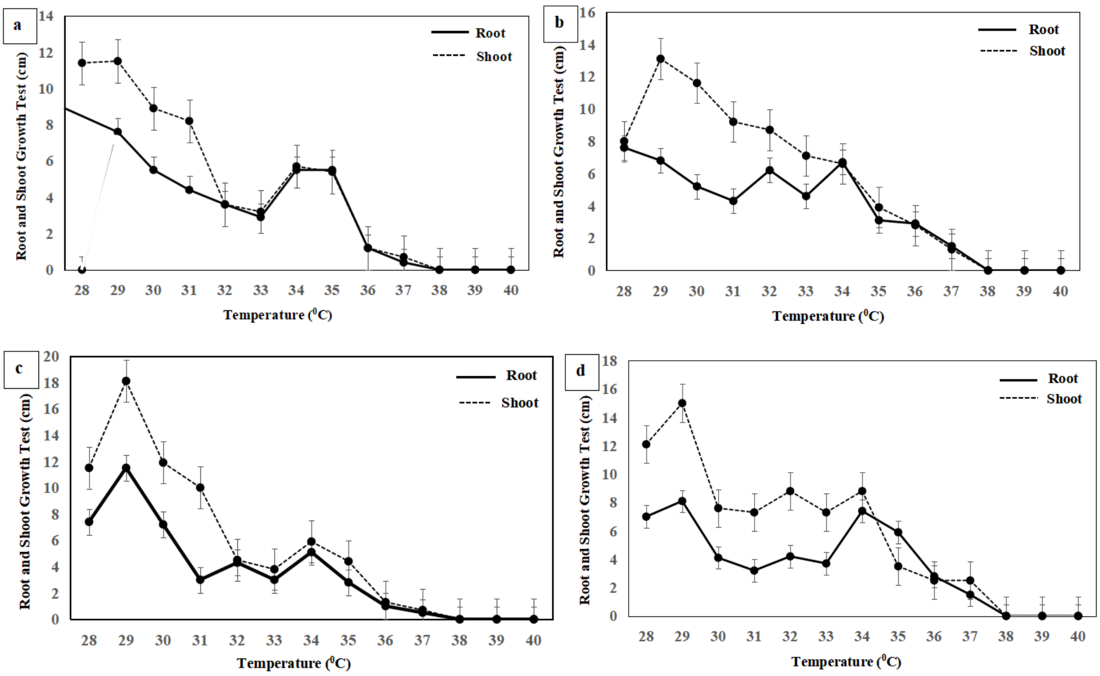
The integrity and functions of biological membranes are sensitive to high temperatures; for example, heat stress alters the tertiary and quaternary structures of membrane proteins (Dongsansuk et al., 2021). Sultana et al.(2015) reported germination on wheat seeds was the highest germination percentage was observed at 240C (98%), and the lowest was at 380C (18%), in the range of 24 to 290C almost all seeds germinated within three days, it was concluded that the optimum temperature for germination of the wheat seeds was a range of 24 to 290C.

Akman (2009) showed that temperature exposure at 35, 38, and 41°C reduced the germination of rice and sorghum. Spears et al. (1997) reported that the high temperature at 38/33°C (day/night) resulted in a low percentage of normal seedlings but a higher percentage of the abnormal seedling. Dongsansuk et al. (2018) also reported that an abnormal seedling in some rice cultivars such as Dular seed was the most tolerant to high temperature, showing the lowest percentage of abnormal seedlings after the seed was exposed to 40°C.

Many physiological changes under high-temperature stress may be reflected in the abnormality of seed germination. Differences in the response of each genotype can be caused by genetic differences in the chemical content of the seeds. Therefore, further observations such as genetics and seed biochemistry are essential. Krishnan et al.(2011) also reported that developing high-temperature stress-tolerant rice cultivars has become a proposed alternative but requires a thorough understanding of genetics, biochemical, and physiological processes to identify and select traits and enhance rice cultivars' tolerance mechanisms.

Root and Shoot Growth Test (cm)

Observations on root and shoot of sprouts were carried out at 14 days after germination by observing and measuring the length of roots and shoots of sprouts using a ruler and thread, which could indicate that the growth of plant organs was strongly influenced by differences in the temperature growing.



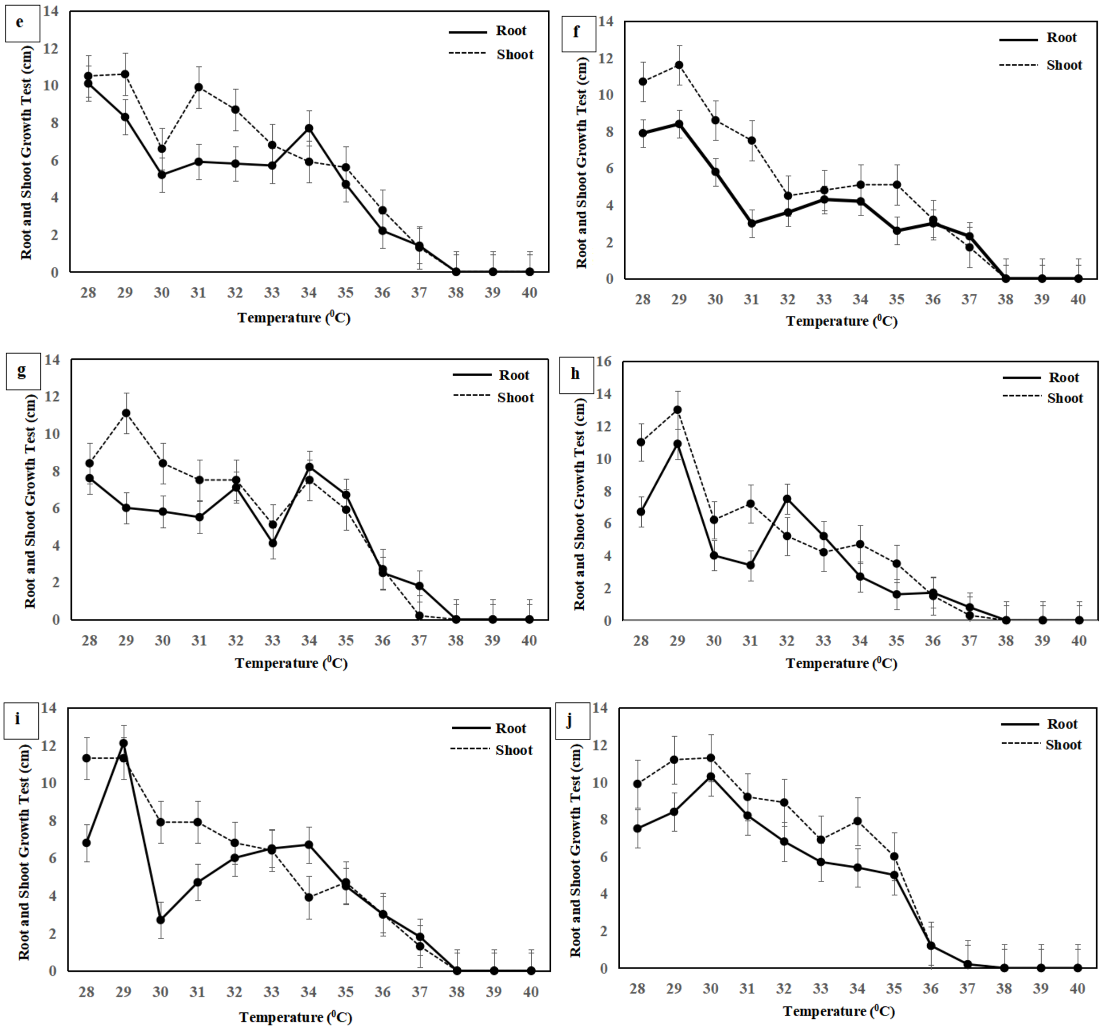


Figure 2. Root and Shoot Growth Test (cm) curve for seeds (a) Pulen Mudiak (b) Pulen Kandih (c) Pulen Marapak (d) Pulen Talao (e) 64 (f) Sibandung (g) Silalang (h) Timbo Abu (i) Labuah Baru (j) Melayu

These experimental results concluded that every 1 ^{0}C temperature increase impacts root and shoot (Figure 2) At temperatures up to 30^{0}C, the mean root and shoot of Melayu decreased significantly. The growth and development of sprouts are very dependent on the supply of nutrients in the endosperm (Sutopo, 1993). Rice has a hypogeal germination type where the emergence of the radicle is followed by elongation of the plumule; the hypocotyl does not extend above the soil surface while the cotyledons are in the seed coat below the soil surface. According to Krishnan *et al.* (2011), the radicle extension of rice sprouts is optimum at a temperature of 30^{0}C and stops at temperatures below 15^{0}C and above 40^{0}C.

In general, changes in stressed plants' high temperatures are grouped into several types morphological changes, anatomical, phenological, and physiological (Wahid *et al.,* 2007). In addition, different phenological phases have different levels of sensitivity to high-temperature stress, depending on the genotype and species, where there is sizeable intra-specific variation. High temperature can damage mesophyll cells and induce increased membrane permeability. Dongsansuk *et al.* (2018) also reported that the integrity and functions of biological membranes are sensitive to high temperatures; for example, heat stress alters the tertiary and quaternary structures of membrane proteins.

Red or brown-colored rice varieties are rich in iron and zinc, while black rice varieties are exceptionally high in protein, fat, and crude fiber. Red and black rice get their color from anthocyanin pigments, which have free radical scavenging, antioxidant capacities, and other health benefits. The differences in genetic makeup and the climatic conditions in which they are cultivated determine the moisture content in rice varieties (Priya *et al.,* 2019).

4. Conclusions

Based on the seeds' quality by observing the seeds' viability and vigor, Pulen Marapak had a maximum growth potential value of 10% at 37^{0}C and high viability and vigor of ten genotypes below 33^{0}C. The optimum germination temperature of the ten varieties tested was at 28-33^{0}C with normal seed germination of

≥80%, a maximum temperature of 37^{0}C, and no seed germination at 38-40^{0}C.

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