Tracking optimum temperature for germination and seedling characterization of three millet (Setaria italica) accessions

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Abstract. The research was conducted at the Plant Physiology Laboratory of Research Center for Biology LIPI. The study aimed to provide information about the prediction of millet plant distribution based on temperature for germination. The experiment was arranged based on factorial RCBD. The treatment consisted of two factors, namely accession (Polman, Gambirmanis, and Enggano) and germination temperature (10, 20, 30, and 40°C), with three replications. The seeds were germinated in Petri dish. Each millet accession had distinctive characters. The color of the de-husked grain affected the color of flour, influencing the quality of end-products. The grains color of Accession Polman was white, Gambirmanis had grey color, and Enggano seeds were yellowish. The optimum germination temperature for three millet accessions was: Polman seeds obtained optimum temperature at 40°C, Gambirmanis possessed optimum temperature at 20 – 30°C, and Enggano had the optimum temperature at 10°C. Millet seeds produced a high percentage of germination and the best in seedlings grown at optimum temperature. At this temperature, accession Polman had the best sprout performance overall. Millet seed germinated at the higher temperature (40°C) appeared to be seized, with a low growth rate, except for Polman. It is assumed that millet would be adapted well in the lowlands to highland based on its germination temperature requirement. However, this plant grew optimally in the lowlands with a germination temperature of 30 - 40°C. Conservation of millet species could be done through seed storage with low temperature and humidity.

1. Introduction
Millet (Setaria italica L.) is a cereal plant. This species originates from the highlands of China [1,2]. Millet was once a staple food in several countries before rice cultivation was known. Only a small portion of local people in Indonesia recognize millet as human food, while the majority still considers this commodity as bird feed. At the meantime, several countries such as China, India, Russia, Africa, and the USA cultivate millet on a large scale to processed food [3]. Millet as a nutri-cereal is very suitable to be nutritious snacks to support food security and anticipate malnutrition [4], including stunting. Local people of the Nangararo sub-district in Flores Island (Nusa Tenggara Timur Province) have known millet plants since a long time ago with the name wete. The seeds of this plant are processed as human food.

Millet is one of the world’s six major cereal commodities, and it is consumed by one-third of the world’s population. Millet-base food serves as the main source of energy, protein, vitamins and minerals, niacin, B6 and folacin as well as essential amino acids such as isoleucine, leucine, phenylalanine, threonine and nitriloside compounds that play a role in inhibiting the development of
cancer cells (anti-cancer), and reduce the risk of heart disease (arteriosclerosis, heart attack, stroke, and hypertension) [5]. Kamatar stated that millet had a carbohydrate content equal to rice and had a higher protein, calcium, phosphorus, iron, and vitamin B1 content than rice [6].

Millet belongs to the Family Poaceae (grass). Plant height reaches 2 m, which depends on the growing environment. The millet panicle comes out at the top of the canopy, cylindrical with dense seeds attached to the panicle with a spiral arrangement. The panicles are similar to a foxtail at a glance. Therefore, S.italica is also named as foxtail millet. The flower is a hermaphrodite, self-pollinated. The flowers in the lower part of the panicle are sterile, while the upper part is hermaphrodite. Millet grain belongs to tiny seeds (3 mm in diameter) [7], which are ovoid and attach to petal husks, and crown husks. It is pale yellow, orange, red, brown, or black when ripe.

Millet is a C4 plant type that adapts well in arid and semiarid areas [8]. This species also potentially grows well in a wide range of soil textures, starts from sandy soil to clay soil. Even this plant can still grow on rocky soils on the hillside in East Sumba. Besides, millet is also reported being able to adapt to various environmental stress conditions such as drought stress [9, 10] and salinity stress [9, 11]. The optimum and maximum growing temperature of sorghum and millet are reported higher than other cereal plants when they enter certain growth stages.

Besides water content, the temperature is an important requirement for seed germination. In the seed germination, there are three critical temperature levels (cardinal germination temperature), namely minimum, optimum and maximum temperature [12]. The minimum temperature is defined as the temperature range, where the seeds can germinate 50% of the population of a seed lot. The optimum temperature is the temperature at which germination occurs earlier, with the highest percentage of seed germination. In the meantime, the maximum temperature is the highest temperature for 50% of germinated seeds [12]. Temperature above the maximum temperature causes seed metabolism to be damaged and die [13]. Information about cardinal temperature, especially for Indonesian millet accessions, for S.italica germination has not been widely reported even though it has been cultivated.

The research aims to explore information about the unique characteristics of the three accessions of S.italica collection from the Research Center for Biology LIPI and determine the range of optimum temperatures in millet germination. The data are the basis for estimating the distribution of millet in nature based on altitude.

2. Method
The study was conducted at the Plant Physiology Laboratory, Research Center for Biology, LIPI, Cibinong. The research material was LIPI collections, namely Polman, Gambirmanis, and Enggano. Polman and Gambirmanis accessions were harvested from the garden collection of the Research Center for Biology, LIPI, while Enggano accession was obtained from exploration to the Enggano Island, Bengkulu Province. The elders of both Polman and Gambirmanis came from Poliwalimandar (West Sulawesi) and Gambirmanis (Central Java) areas.

The experiment was arranged in a factorial RCBD (Randomized Complete Block Design) design, consisting of two factors and three replications. Each replication consisted of 100 millet seeds. The first factor was the germination temperature consisting of four levels, namely 10, 20, 30, and 40 °C. The second factor was accession, i.e., accession Polman, Gambirmanis, and Enggano. Replication blocks in the germination chamber were arranged based on placement on the shelves, while block in the screenhouse was based on the direction of the sunshine.

The moisture content and viability of the seeds were measured before the treatment. The seeds of each accession were planted in the petri dish with moist tissue towell media. Seeds were germinated in the germination chamber for eight days in the dark condition. One germination chamber was for each treatment. Petri dish was closed to keep the media moist. The humidity of treatment media reached 100%. Watering was done by spraying aquadest two times a day. On the 8th day after the seeds germinated, seedlings were planted into a tray with a mixture of soil and manure (1: 1 v/v) as the media. The seedling was then stored in the screenhouse. Daily temperature in the screenhouse was around 25°C with 55% humidity in the morning, while temperature reached 37°C with 50% humidity.
at noon. Watering in the tray was done once a day, while the management of pest and disease (OPT) was carried out if necessary.

Seeds moisture content was measured by the gravimetric method. Then, the seeds were dried using 100°C for three days. The tetrazolium test was conducted using the following direction. Ten seeds per replication of each accession were split until the embryo part was exposed. 1% tetrazolium solution was dripped onto the seed surface and incubated overnight. The bright red color in the embryo part means the seeds were alive. In contrast, the pale red until white indicated that the seed was unviable.

Characterization was conducted both on seeds, 8-day-old sprouts, and seedlings. Exclusively for sprouts level, we only characterized the sprouts that grew at optimum temperature. Seed characterization referred to millet UPOV [14], including seed shape, grain color, de-husked seed color, and seed size. Observation variables of germination included germination rate, number of normal sprouts, radicle length, plumula length, and sprout biomass. At the same time, the variables at a seedling level were seedling height, number of leaves, and fresh root weight.

Data were presented in the form of images, tables, and histograms. ANOVA test with Duncan multiple tests at a 5% significance level was employed to analyze the data. The analysis was performed using SAS statistical software for Windows v 9.0.

3. Results and discussion
Genotype-based characteristics influence seed vigor on a diver’s germination environment [15]. For that reason, in this study, seed characteristics were determined before the seed was planted at different germination temperature. The effect of temperature on seed was observed in several parameters, namely variables on pre-germination (seed viability and water content), germination (germination rate, biomass sprout, and sprout size), and post-germination (seedling height, number of leaves, and fresh root weight). Each phase is presented as follows.

3.1. Characterization of millet
In this study, the characterization was done at seeds and sprouts level. The parameters were seed and sprout morphology with several variables such as the color, size, and shape of the seeds and sprouts [14] (Table 1).

| No | Variables                 | Polman | Gambirmanis | Enggano |
|----|---------------------------|--------|-------------|---------|
| 1  | Coleoptile color          | Green  | Green       | Green   |
| 2  | First foliage leaf color  | Green  | Green       | Green   |
| 3  | Brash root color          | White  | White       | White   |
| 5  | First foliage leaf length | 10-12 mm | 9.5-11 mm | 8.5-11 mm |
| 6  | First foliage leaf width  | 2-2.5 mm | 2-3 mm     | 1-2.5 mm |
| 7  | First foliage leaf shape  | Long slim | Long slim | Long slim |
| 8  | Grain color               | Yellowish | Grey-yellow | Yellowish |
| 9  | 1000 seed weight          | 1.498 g  | 1.735 g     | 1.318 g  |
| 10 | De-husked grain color     | White  | Grey        | Creamy   |

The three accessions were similar in several characters, namely the color of the first foliage leaf (green), coleoptile color (green), brash root color (white), and the shape of the first foliage leaf (long slim). Moreover, the size of the first foliage leaf differed among three millet accessions. The first foliage leaf in Enggano Accession was the shortest (8.5-11 mm) and narrowest (1-2.5 mm) compared to Polman (10-12 mm; 2-3 mm) and Gambirmanis (9.5-11 mm; 2-3 mm) at the same age. The grain of Polman accession and Enggano was yellow, while Gambirmanis was greyish yellow. The de-husked grain color for Polman accession was white, while Gambirmanis was grey, and Enggano had yellowish-white color. The color of the de-husked grain greatly influenced the flour color, which affected the appearance of the final millet product. Thus, the color of the seeds indirectly affected consumer preferences for millet-based products. The weight of 1000 seeds of the Enggano accession...
was around 1.318 g, the accession of Gambirmanis was around 1.735 g, while Polman 1.498 g (Figure 1).

![Figure 1](image.png)

**Figure 1.** Characters of panicle, seed, and sprout of each millet accession.

3.2. Pre-germination

The seed viability observation was conducted by the tetrazolium test in this study. This test has been developed according to the capability of tetrazolium to stain the living parts of a seed [16]. The red color in all parts of the seed or all parts of the embryo is declared as viable seed [17].

The tetrazolium test on three millet accessions showed that red color was produced in all parts of the seeds, both endosperm and embryo parts (Figure 2). One hundred percent of the sample test from Polman showed that all seeds were alive. In Gambirmanis, 93% of the sample test was viable, while Enggano seed, only 33% were alive (Table 2).

![Millet seed before dripped by 1% tetrazolium solution](image.png)

Millet seeds after dripped by 1% tetrazolium solution

The appearance of embryo and endosperm, after dripped by 1% tetrazolium solution

**Note:** The test was carried on three millet accessions. Ten seeds in every replication of each accession were dripped with a 1% tetrazolium solution and incubated overnight. The bright red color in the embryo part meant the seeds were alive. In contrast, the pale red until white indicated that the seed was unviable.

**Figure 2.** The performance of millet seeds using the tetrazolium test. The observation was done using a light microscope with 10 x 4 magnification.

The tetrazolium test can identify the activity of the dehydrogenase enzyme in the embryonic tissue of the seed [18]. Besides, the test can identify tissue viability [19]. Tetrazolium test is an indirect
method for viability tests. Therefore, the correct data of the tetrazolium test must be proven through the germination test. The basic principle of the tetrazolium test is a color change of the 3,3,5-Triphenyl tetrazolium chloride compound. It changed from colorless to red formation after interacting with the seed starch part.

The water content of the seed was measured to explore the cause of the low percentage of live seed in Enggano accessions. The water content of the seed can describe the condition of seed metabolism, so it is suspected, causing the low viability of the seed. The test results showed that the water content of seed from three millet accessions was around 8-9% (Table 2). The lowest water content was obtained for the Polman, followed by Gambirmanis, and Enggano. Millet seeds belong to the orthodox group [20], which needs to be stored at low moisture content. Seed with low moisture content tends to be lower in metabolism activities and can be stored for hundreds of years, without reducing its viability.

Otherwise, the results of this study indicated that storage at 9% water content could sufficiently reduce seed viability for Enggano accession; even the three accessions had a similar storage environment for one year after being harvested. The difference of seeds moisture content in this study was influenced by the genetic factor of each millet accession and environment of mother plant habitat (seed source).

### Table 2. Seed water content and tetrazolium test.

| Accession | Polman | Gambirmanis | Enggano |
|-----------|--------|-------------|---------|
| Water content (%) | 8.84   | 8.57        | 9.06    |
| Viable seed (%)     | 100.00 | 93.33       | 33.33   |

#### 3.3. Germination

The germination process started from the imbibition of water into the embryo through the seed coat followed by plumula and radicle, then it grew into a young plant (seedling) [21,22]. In the present study, the percent of germination, radicle length, plumula length, number of leaves, and the sprouts biomass at eight days after germination were also observed.

### Table 3. Sprout performance on 8th day after germinating on different temperature.

| Temperature (°C) | Germination rate (%) | Radicle length (mm) | Plumula length (mm) | Number of leaves (strands) | Water content of sprout (%) |
|------------------|----------------------|---------------------|---------------------|---------------------------|-----------------------------|
| 10               | 87.33 a              | 14.82 c             | 14.26 d             | 2.67 b                    | 81.10 c                     |
| 20               | 81.33 a              | 30.14 a             | 36.74 b             | 3.00 a                    | 91.16 b                     |
| 30               | 86.33 a              | 29.96 a             | 65.33 a             | 1.00 c                    | 92.70 a                     |
| 40               | 65.57 b              | 19.55 b             | 27.74 c             | 1.00 c                    | 91.26 b                     |

Note: *) The same letter in each column were not significantly different based on Duncan’s test of 5%.

Table 3 shows that every millet accession gives different responses from the effect of temperature on seeds germination. Polman seeds obtained a high percentage of germination in all ranges of temperature. However, the highest (99.67%) was found on seeds sown at temperature 40°C. Gambirmanis seeds would obtain germination around 82.67% if seeds were sown at temperature 20 – 30°C. When Gambirmanis seed sown at temperature 10°C and 40°C, it produced a lower percentage of germination. Enggano seeds produced a high percentage of germination, which was around 98.33% if seeds were sown at temperature 10°C. In comparison, seeds sown at temperature 20 – 40°C obtained a lower percentage of germination, around 33.33 – 62.33%. The difference in germination percentage from three accessions of millet was influenced by temperature. This case occurred due to the enzyme temperature-controlled activities contributing to the process of seed germination. The result of this study showed that Polman seeds needed a temperature of 40°C as optimum temperature, Gambirmanis seeds needed a temperature of 20-30°C as optimum temperature, and Enggano seeds needed a temperature of 10°C as optimum temperature. In the final observation, the Enggano accession had
98.33% of seed germination at 10°C. While Polman had 99.67% of seed germination at 40°C, Gambirmanis had 82.67% of seed germination at 20°C and 30°C.

**Figure 3.** Comparison of germination speed from each accession at different temperature.

The germination rate did not differ among treatments until the temperature reached 30°C, while the lowest percentage of germination at 40°C (65.57%) was different from all temperature treatments (Figure 3). Data showed that the temperature of 10 - 30°C was suitable for the germination of millet seed (Table 3). Figure 4 shows a comparison of the radicle length of each
treatment combination, and Figure 5 illustrates the comparison of the Plumula length variable.

After germination, the temperature of 10°C caused the slow growth of sprouts (Figure 4 and 5). The sprout germinating at 10°C produced the shortest radicle (14.82 mm) and plumula (14.26 mm), followed by 40°C (19.55 mm; 27.74 mm). The values were different from the temperature of 20°C (30.14 mm; 36.74 mm) and 30°C (29.96 mm; 65.33 mm) (Table 3).

Figure 5. Plumula growth each accession at different temperature.

The seeds germinating at 20°C (3.00 strands) had the most leaves of all temperature treatments, while 30°C and 40°C had a lower number of leaves. The highest water content of sprouts was at 30°C (92.70%), while the lowest one was at 10°C (81.10%). Based on these results, it can be suggested that the temperature of 20 - 30°C is suitable for millet seed germination. The lower temperature made lower metabolism of the sprout, so sprout grew slower. Whereas higher temperatures caused plant metabolism to run faster, and seed energy ran out for growth. However, the roots were not ready to look for enough nutrients for the shoot growth, which resulted in plant growth slowing down (Table 4).

Table 4. Sprout performance on 8th day after germinating on different accession.

| Accession   | Germination rate (%) | Radicle length (mm) | Plumula length (mm) | Number of leaves (strands) | Water content of sprout (%) |
|-------------|----------------------|---------------------|---------------------|---------------------------|---------------------------|
| Polman      | 98.92 a              | 26.47 a             | 38.08 a             | 2.00 a                    | 89.21 a                   |
| Gambirmanis | 75.50 b              | 26.67 a             | 35.94 ab            | 1.92 a                    | 89.27 a                   |
| Enggano     | 66.00 c              | 17.72 b             | 34.03 b             | 1.83 a                    | 88.69 a                   |

Note: *) The same letter in each column was not significantly different based on Duncan’s test of 5%.

Each accession had a similar response to temperature, but there were some adaptation rates (Table 5). Polman had a higher adaptation to germination temperature. This accession had the highest germination percentage (98.92%), longest plumula (38.08 mm), and the highest number of leaves (2.00 strands). Meanwhile, Enggano was less adaptable for germination temperature, since it had the lowest growth at almost all variables, namely germination percentage (66.00%), radicle length (17.72 mm), plumula length (34.03 mm), number of leaves (1.83 strands), and water content of sprouts (88.69%) (Table 4). The low growth of Enggano was due to higher seed moisture content. With a higher water content, the respiration rate is higher, so that the energy used for growth is less than other accessions.

All millet accessions have good germination. At an optimal germination temperature of 30°C, Polman accession had the highest germination rate. Almost 100% seed population of this accession had germinated since the 4th day. Gambirmanis came next, followed by Enggano. At lower
Germination temperatures (10°C), Polman still could maintain a high germination rate and germination speed (Figure 3). In contrast, germination speed values for Gambirmanis and Enggano accessions were reduced by half, on the 8th day. The same experiences were found in the temperature of 40°C. Enggano seemed to be more vulnerable to temperature rise (Table 5).

Table 5. Temperature x accession interaction on millet seed germination.

| Temperature (°C) | Accession | Germination rate (%) | Radicle length (mm) | Plumula length (mm) | Number of leaves (strands) | Water content of sprout (%) |
|------------------|-----------|----------------------|---------------------|---------------------|---------------------------|---------------------------|
| 10               | Polman    | 98.67 a              | 21.22 cd            | 20.33 e             | 3.00 a                    | 79.47 d                   |
|                  | Gambirmanis | 73.67 cd          | 13.45 ef            | 13.00 f             | 2.67 ab                   | 79.99 d                   |
|                  | Enggano   | 98.33 a              | 9.78 f              | 9.45 f              | 2.33 b                    | 83.83 c                   |
| 20               | Polman    | 99.00 a              | 33.67 ab            | 40.33 c             | 3.00 a                    | 91.62 a                   |
|                  | Gambirmanis | 82.67 bc          | 38.22 a             | 40.78 c             | 3.00 a                    | 91.58 a                   |
|                  | Enggano   | 62.33 d              | 18.56 de            | 29.11 d             | 3.00 a                    | 90.29 ab                  |
| 30               | Polman    | 98.33 a              | 30.11 b             | 63.11 b             | 1.00 c                    | 92.92 a                   |
|                  | Gambirmanis | 82.33 bc          | 35.45 ab            | 60.55 b             | 1.00 c                    | 92.92 a                   |
|                  | Enggano   | 78.33 bc             | 24.33 c             | 72.33 a             | 1.00 c                    | 92.26 a                   |
| 40               | Polman    | 99.67 a              | 20.89 cd            | 28.55 d             | 1.00 c                    | 92.84 a                   |
|                  | Gambirmanis | 63.33 d          | 19.55 cd            | 29.45 d             | 1.00 c                    | 92.58 a                   |
|                  | Enggano   | 33.67 e              | 18.22 de            | 25.22 de            | 1.00 c                    | 88.37 b                   |

Note: *) The same letter in each column was not significantly different based on Duncan’s test of 5%

Observation of seed vigor included the ability of the seed to germinate and sprouts performance. Figures 5 and 6 illustrate the growth after germination. All three accessions showed a uniform response to germinating temperature. The sprout growth was inhibited when the seed germinated at a temperature of 10 °C, both radicular and plumular. The temperatures of 20 °C and 30 °C increase germination growth because of the increase in plant metabolism. Then, plants can no longer maintain metabolism when the seed germinates at 40 °C. Therefore, growth decreases (Figure 6 and 7).

Figure 6. Sprout performance of each accession on two days (left) and four days (right) after germination.

Polman had higher growth, followed by Gambirmanis, while Enggano had the lowest growth, especially at temperatures of 10 °C and 40 °C. It has been suggested that Enggano is sensitive to an...
increase or decrease in germination temperature (Table 5, Figure 6 and 7). The ability of Polman to balance shoot and root growth, in low and high temperatures indicates that this accession has broad adaptation in terms of temperature for germination, as [23] report at rapid selection for low temperature in paddy. It can be suggested that this accession is the potential to be distributed or planted in broader ecosystem types in Indonesia.

![Figure 7](image7.png)

**Figure 7.** Sprout performance of each accession on six days (left) and eight days (right) after germination.

According to Sadjad, the metabolic rate for seed growth can be expressed in three stages, namely reshuffle food reserves, nutrients translocation from one part of the seed to other parts, and synthesis of new materials [24]. Sutopo explains the stages of the germination process as follows: The first stage begins with the absorption of water by the seed, softening the seed coat, and protoplasm hydration [17]. The second stage starts with the activity of cells and enzymes and the increase in seed respiration rate. The third stage sets to carbohydrate, fat, and protein decomposition into smaller molecules that can dissolve and transport to growth points. The fourth stage is the assimilation stage of decomposed materials that are intruded into the meristematic region to produce energy for new cells. The fifth stage is the growth of sprouts through the process of division/enlargement and differentiation of cells at growing points.

![Figure 8](image8.png)

**Figure 8.** The number of leaves in seedling for up to 8 days after being transplanted.
3.4. Growth of millet seedling

There was a change in the growth behavior of the seedling after being transplanted into polybags. Plants germinated at a temperature of 10 °C to 20 °C were able to grow after being transplanted into polybags. Meanwhile, plants that were grown at 30 °C and 40 °C all died, so as there were no data collected from both treatments (Figures 8 and 9). It is suspected that the seedlings ran out of growth energy, so it died when it was transplanted to polybag. The seed ran more respiration activity when it sowed in high temperature. At the same time, the plants had not yet obtained nutritional intake from the outside because the roots were not in contact with the soil (germination was carried out in tissue media in petri dish). The seed that germinated at 30 °C and 40 °C resulted in etiolated sprouts. It made it difficult to adapt to temperatures in the screen house.

![Figure 9. The seedling height for up to 8 days after being transplanted.](image)

4. Conclusion

It can be concluded that temperature affects the seed germination and the sprouts’ performance of the three millet accessions. We strongly suggest that there are three kinds of optimal temperature for three millet seeds accession, which are: Polman seeds obtained optimum temperature at 40°C. While Gambirmanis seeds possessed optimum temperature at 20 – 30°C, Enggano seeds had the optimum temperature at 10°C. Based on these data, it can be suggested that millet can be planted from the lowlands to the highlands depending on millet accession. Polman accession had a wider range for distribution than Gambirmanis and Enggano. In the highlands, millet seeds could germinate but had relatively slow growth and lack vigor. Compared to the Gambirmanis and the Enggano, Polman’s accession had a wider adaptation to germination temperature. Polman had a pretty good tolerance for temperature increases and decreases at the germination level. The color of de-husked millet seeds affected the appearance of seed millet-based products. Polman accessions had white seeds. While Gambirmanis accessions had grey seeds, Enggano accessions seeds were yellowish-white. The use of these three accessions on processed products can be adjusted to consumer preferences. Conservation of millet species can be done through seed storage with moisture content was lower than 8%-9%.

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