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Harvesting Criteria and Drying Methods to Improve the Quality of Foxtail Millet Seeds (*Setaria italica* L. Beauv.)

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Abstract

Foxtail millet is annual grasses with grains that are smaller than those of sorghum, rice, and wheat, and is considered one of the minor economic crops but with nutritional values similar to other major food crops. The development of millet as major cereal crop is considered challenging due to the low quality of its seeds, and studies have been conducted to improve millet's seed quality. We conducted this study to determine the harvesting criteria of foxtail millet seeds based on the change in color of the panicles. We also wanted to determine the drying treatment, and evaluate the relationship between the position of the seed on the panicles and the seed viability and vigor. The first experiment was arranged based on a completely randomized design with panicle color as the first factor (green, yellow 75%, and brown) and drying time as the second factor (0 hours, 24 hours, 48 hours, 72 hours). The second experiment was arranged in a completely randomized design with seed position as the main factor (base, middle, tip). The study was conducted on two genotypes of millet, i.e. "BOTOK 4" and "BOTOK 10". The highest seed quality of "BOTOK 4" and "BOTOK 10" genotypes were obtained when the panicles were brown and dried for 72 hours; the seed chlorophyll content was the lowest and water content was 8.88%, with the highest viability and vigor, i.e., germination rate of 88.3%-90%, maximum growth potential of 92%-95.3%, normal sprout dry weight 596-620 mg, vigor index of 33.6% - 21.6%, and growth rate of 18.2%-17.1% etmal^{-1}. The highest seed viability and vigor in "BOTOK 4" genotypes were obtained from the base position, i.e., 78.7% germination rate, maximum growth potential of 83.7%, vigor index of 56.5%, growth speed of 19.6 etmal^{-1}, normal sprout dry weight of 48 mg, radicle length of 3.3 cm) and "BOTOK 10" genotypes from the middle position with 91.5% germination, maximum growth potential of 97.2%, vigor index of 21.7%, growth speed of 17.0% etmal^{-1}, and normal sprout dry weight 61 mg.

Keywords: chlorophyll content, germination, seed vigor physiological seed quality, seed position,

Introduction

Foxtail millet (*Setaria italica* L. Beauv.) is one of the cereals mainly consumed by the people of the island of Buru, Maluku. Foxtail millets are monocots that belongs to the family of Poaceae or grasses. Foxtail millet "BOTOK 4" genotypes has a round seed shape with a bright yellow color. Another genotype of foxtail millets, "BOTOK 10", has an egg-round seed shaped with a medium yellow color (Zahroh 2017). Studies on agronomic performance of millet have been carried out by Bogor Agricultural University (IPB) research group, and there are variations in the potential production and important morphological and agronomic traits in the millet genotypes.

Indonesia has diverse biological resources, allowing it to obtain foods from many other species besides rice. One of the potential food sources alternative is millet (*Setaria italica* L. Beauv). Foxtail millet has a low glycaemic index and has been reported to have anti-cancer properties (Saleh et al. 2013). Studies have shown that the low glycaemic index may result in weight loss, reduce blood sugar levels, and lower the risk of heart disease and type 2 diabetes (Sacks et al. 2014). It also contains high phenolic compounds, flavonoids and antioxidants (Sharma et al. (2015) germination time (Gt. Millet from various regions in Indonesia contains carbohydrates of 72% - 84.2%, protein of 9.9% - 12.07%, fat of 2.38% - 4.90%, crude fibre of 1.4% - 10%, and several important minerals (Tirajoh, 2015). According to FAO (2019), global millet production is estimated at 28.4 million m^3. The commonly available genotypes in Indonesia, "BOTOK 4" and "BOTOK 10", use yield protection of 3.4-3.5 tonnes.ha^{-1} (Zahroh, 2017).

The yield potential of these two genotypes is still low, i.e. 2.51–2.61 ton.ha^{-1} (Zahroh, 2017). One of the main obstacles in the cultivation of millet is the low quality of the seeds due to low germination, low plant vigor, and plant disease. Inappropriate harvest time and seeds maturation are also one of the causes of low seed quality. Bishaw et al. (2007) reported that seed quality comprises many aspects where four key attributes are explicitly identified: genetic, physical, physiological and health quality. However, seed quality can be affected by environmental conditions under which the crop is grown and the cultural practices used for production (Bishaw et al., 2007). The average germination of millet seeds is 56.67% (Simanjuntak, 2012) and less than 70% (Sugri et al., 2011). Ardie et al. (2015) also reported that four accessions (ICERI-1, ICERI-2, ICERI-3, and ICERI-6) had germination percentage <80% but still >70% under unstressed condition. Seeds of high quality can be obtained by planting in suitable areas and at appropriate times, applying good crop management practices, adoption of proper harvesting and drying techniques, careful handling and processing to minimize mechanical injuries and unwanted seed mixing with other accessions, and ensuring minimum deterioration before reaching the designated storage (Nanduri et al., 2016). High quality seeds, especially physiological qualities, can be attained if seeds are harvested at physiological maturity. Seeds at physiological maturity usually have maximum dry weight, viability and vigor (Murniati and Sari, 2008) and maximum quality (Ichsan et al., 2013). The criteria for harvesting high quality seeds can include colour of the seed or fruit coat, and size and shape of the fruit (Mutiarawati, 2007).

Millet is an orthodox seeds, i.e. seeds that are can be dried to reach the internal seed moisture of < 12%, stored at freezing temperatures, and survived (Solberg et al., 2020), Therefore, a proper method of seed drying after harvesting is required to reduce the seed moisture content to reduce the respiration rate of the seeds and extend the seed's life. Seed drying should reduce the seed moisture content to safe moisture limits and to maintain its viability and vigour during storage, which may otherwise deteriorate quickly owing to mould growth, heating and enhanced microbial activities (Magistrali et al., 2015). Drying seeds at high temperatures of 40°C – 100°C, or 30°C may cause physical damage, contamination, and decreased seed viability (Vijay et al., 2015). The optimum temperature for seed drying are about 25°C – 35°C (Devi and Mani, 2019). Seed drying methods can be used during seed processing, including drying the seeds under the sunlight, using a vacuum or a cooler with 10-15% relative humidity, seed drying chambers, and seed dryers, are recommended (Magistrali et al., 2015). The optimum RH for millet seed storage conditions maintain relative humidity levels between 20% and 40%, giving corresponding seed moisture contents between 5% – 8% (Nascimento et al., 2018).

Seeds of millet are generally sun-dried to achieve a moisture content of 12% (Kannan et al., 2013). The seed moisture content of 3-7% and storage temperature below 0°C are suitable for long-term storage of orthodox seeds. The drying method using silica gel can reduce the water content quite low without damaging the viability and vigour of seeds (Varghese and Naithani, 2008)catalase (CAT. Vijay et al. (2015) showed that the drying method of sorghum seeds takes about 7-11 days to reach a moisture content of 6 ± 0.1%. Silica gel is a drying method that provides the highest drying rate after sulfuric acid with a germination percentage of 79% and can maintain seed quality (Vijay et al., 2015).

Determining and comparing the quality of millet seeds coming from different segments of the panicle (i.e. tip, middle, base) has not been done yet during seed production process. Martinatti et al. (2020) reported that the millet seeds located in different panicles show different seed qualities physically, and physiologically, with the highest quality originating from the middle position. Based on observations in the field the elongated panicle structure with dense seeds, especially in the "BOTOK 10" and "BOTOK 4" genotypes, has different levels of maturity from the base to the tip of the panicle.

The quality of millet seeds can be improved by harvesting the seeds from the particular segment of panicles using specific panicle colour criteria and efficient drying method. It has been shown that among cultivated plants, the ripening phase is determined by the loss of pigmentation in the parent plant and a change in colour in the seed coat (Ilyas, 2012). This study aims to optimise the protocols for generating high quality millet seeds by a) identifying harvesting criteria based on the colour and position of panicles and b) determining the optimal time and techniques of seed drying.

Material and Methods

The research was carried out from October 2019 to September 2020 at Cikabayan Experimental Garden, IPB University, with an altitude of ± 240 m above the sea level, latosol soil type, and climate type A (Schmidt-Ferguson classification). Physiological quality testing of seeds was carried out at the Laboratory for Storing and Testing of Seed Quality. The destructive measurement of seed chlorophyll and

carotenoids was done at the Postharvest Laboratory. All laboratories belongs to the Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University.

Determination of Harvesting Criteria and Drying Method

A total of 72 experimental units, representing 36 for "BOTOK 4" genotype set-ups and another 36 for "BOTOK 10" genotype set-ups, were prepared using a completely randomized design environment. The experimental set-up involved two factors namely panicle colour (F) and drying time (W). Three panicle colours were used i.e. green (90-94 DAS) (F1), 75% yellow (102-105 DAS) (F2), and brown (110-115 DAS) (F3). Four drying times were used: 0 hours (W1), 24 hours (W2), 48 hours (W3), and 72 hours (W4). Out of these two factors and levels within factors, 12 treatment combinations were done with three replicates.

After harvesting panicles from the set-ups, the seeds were extracted using a sorghum threshing machine, and were separated from dirt using a blower and sieve until they were clean. The chlorophyll and carotenoid content from the seeds were measured using standard technique as described in Sims and Gamon (2002). This was a destructive method involving a UV-VIS spectrophotometer (Shimadzu UV-1280). Seeds according to the treatment separated by 0.5 g. Seed chlorophyll testing was carried out using the maceration technique (soaking with acetris solvent for 3-24 hours). The solution was homogenized and collected in a tub, then filtered and centrifuged at 5000 rpm for 7 minutes. The supernatant was formed then added to the test tube. The supernatant was read and the absorbance value was measured with UV-VIS spectrophotometer at wavelengths: 470 nm, 537 nm, 647 nm, and 663 nm (Sims and Gamon, 2002). Absorbance results then put into the formula. The chlorophyll test could be calculated using the calculation formula:

Chlorophyll A = 0.01375 x A663-0.000897 x A537-0.003046 x A647
Chlorophyll B = 0.02405 x A647-0.004305 x A537-0.005507 x A663
Chlorophyll total = Chlorophyll A + Chlorophyll B
where:
A 663 = Absorbance value at a wavelength of 663 nm
A 647 = Absorbance value at a wavelength of 647 nm

Testing of carotenoids was calculated using the calculation formula:
Chlorophyll A = 0.01375 x A663-0.000897 x A537-0.003046 x A647
Chlorophyll B = 0.02405 x A647-0.004305 x A537-0.005507 x A663
Anthocyanin = 0.08173 x A537-0.00697 x A647-0.002228 x A663
Carotene (µmol/ml) =
A470 – [17.1x(Chlorophyll a + Chlorophyll b) – 9.475 x Anthocyanin]/119.26
where:
A 470 = Absorbance value at a wavelength of 470 nm
A 537 = Absorbance value at a wavelength of 537 nm
A 663 = Absorbance value at a wavelength of 663 nm
A 647 = Absorbance value at a wavelength of 647 nm

On the other hand, the seeds were weighed and put in a cloth bag with 25 cm L x 25 cm W x 25 cm H for drying treatment. The drying treatment was carried out in a desiccator containing 100 g of silica gel (Fernandes, 2019). The desiccator was made of a plastic box container measuring 21 cm L x 14.5 cm W x 12 cm H. The temperature and RH during drying were measured by placing a thermohygrometer in the desiccator. The cloth bag containing the seeds were placed in the desiccator and covered with a plastic box. The seeds were dried according to the drying time, i.e. 0 hours, 24 hours, 48 hours, and 72 hours.

The seed moisture content was measured at each drying time using the direct method of high temperature 130 ± 3°C for 1 hour using 0.5 g of seed (duplo) (ISTA, 2018). The viability variables observed were germination (determined based on the percentage of normal sprouts on the 4th and 10th day after germination) (ISTA, 2018), maximum growth potential (based on the number of seeds that germinated normally and abnormally until the final count, which was the 10th day after germination), and normal sprouts dry weight (determined based on normal sprouts on the final count, which was the 10th day, then oven-dried at 60°C for 3 x 24 hours) (Tresniawati et al., 2014). The vigour variables observed were vigour index (determined by calculating the percentage of normal sprouts on the first count, which was the 4th day after germination) and growth rate (observed based on the percentage of normal sprout at each observation time, which was on the 2nd until 10th day after germination) (Tresniawati et al., 2014). The seed viability and vigour tests were carried out at each drying time with 100 grains per replication with plated paper test method using filter paper. Each paper substrate was planted as many as 100 seeds using a seed germination container measuring 17 cm L x 8 cm W x 5 cm H at room temperature (25±5°C), and RH of 80%.

Data Analysis

Seeds from the base (S1), middle (S2), and tip (S3) of panicles that were harvested were compared for viability and vigour. The experimental data were analysed as follows: (1) analysis of variance using SAS 9.4. (2020) on chlorophyll and carotenoid content, moisture content, germination, vigour index, maximum growth potential, growth rate, normal sprouts dry weight, (2) to determine the effect of each treatment further tests were conducted using the Duncan's Multiple Range Test with a test level of 5%.

Results and Discussion

Seed Pigmentation in "BOTOK 4" and "BOTOK 10" Genotypes

The variation in colour and appearance of the seed coat depends on the concentration of pigments such as chlorophyll and carotenoids. Shen et al. (2015) reported that the yellow pigment of some foxtail millet, particularly the Chinese cultivars, contains two compounds of carotenoid, lutein and zeaxanthin. Chlorophyll, carotenoids, and anthocyanin content in millet seeds from "BOTOK 4" and "BOTOK 10" genotype is presented in Figure 1. The seed chlorophyll content in was found to be decreasing, while the carotenoids and anthocyanins were increasing during seed maturation as manifested in the change of panicle color from green to brown. According to Hörtensteiner (2006) chlorophyll in seeds degrades very quickly, and the amount of carotene is stored in a certain amount depending on the species and environmental factors. Suhartanto (2003) also reported that chlorophyll content in tomato seeds decreases and the carotenoid increases as the fruit matures. Carotenoids may come in the form of carotene and xanthophyll. Carotene is the pigment that causes the orange colour, while xanthophyll produces the yellow colour. Xanthophyll levels vary depending on cultivar, landraces, and harvest time. Xanthophyll gradually increase during seed ripening process but decrease rapidly in response to over-ripening of seeds (Yano et al., 2017). Smolikova et al. (2011) reported that carotenoids in seeds are present in amyloplasts and elaioplasts. Asharani et al. (2010) also reported that carotenoids in several Indian finger millet varieties (including some foxtail millet and proso millet varieties) function as antioxidants. Zhang et al. (2014) detected the presence of lutein and zeaxanthin in proso millet seeds. Lutein and zeaxanthin contribute to the yellow and red colour of the millet seeds, respectively.

Seed Vigor and Viability of "BOTOK 4" and "BOTOK 10" Genotypes

The average daily temperature during the study ranged from 25.6°C-27.2°C (BMKG 2020). Millet seeds of "BOTOK 4" genotype has a harvesting age of about 107± 5 DAS and "BOTOK 10" about 110±5 DAS. The recapitulation results of variance showed that panicle colour and drying time had a very significant effect on viability and vigour of the seeds; brown seeds have higher viability and vigour than green and yellow seeds (Table 1 and Table 2). The drying of green panicles had significantly reduced the moisture content of the seeds, but not their viability and vigour. Drying the seeds up to 72 hours can reduce the moisture content from 13.3% to 8.8% for "BOTOK 4", and 14.1% to 8.8% for "BOTOK 10". The seeds harvested from brown panicles had the highest viability and vigour when dried for 48 to 72 hours. This is achieved when the moisture content is 8.8-9.5%, so that drying with this moisture content produces the best viability and vigour. Drying of the

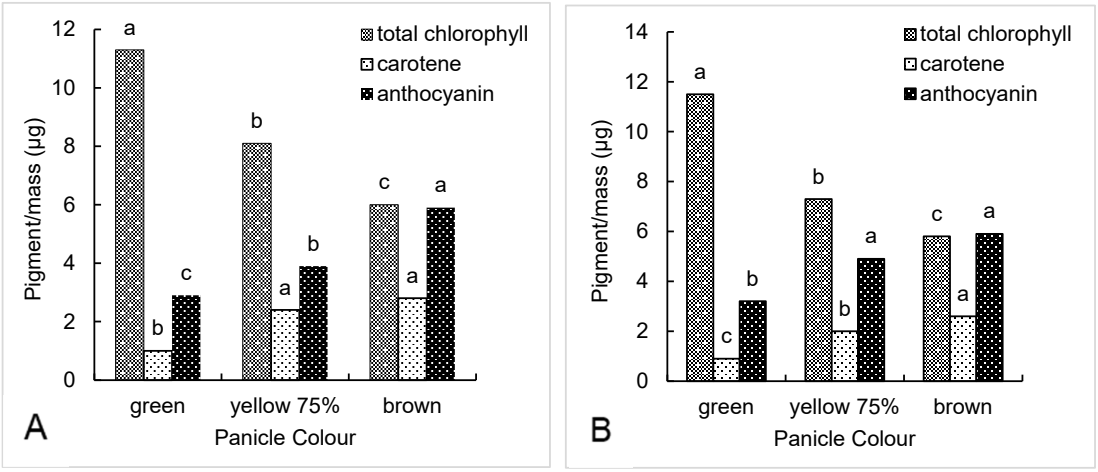

Figure 1. The levels of chlorophyll, carotene and anthocyanins in "BOTOK 4" (A) and "BOTOK 10" (B) genotypes. The same letter in each panicle colour was not significantly different according to DMRT at α = 5%

Table 1. Effect of harvest criteria based on panicle color and drying time on seed viability and vigor of "BOTOK 4" genotypes

Drying time (hour)	Panicle color		
	Green	Yellow 75%	Brown
Moisture content (%)			
0	13.33 a	12.91 a	11.88 b
24	12.22 b	10.87 cd	10.55 cd
48	11.22 c	10.22 d	9.55 e
72	9.33 e	9.00 e	8.88 e
Germination (%)			
0	0.66 e	46.33 d	59.33 c
24	1.66 e	57.66 c	72.33 b
48	2.66 e	70.66 b	85.66 a
72	3.00 e	73.00 b	88.33 a
Maximum growth potential (%)			
0	0.66 e	50.66 d	63.66 c
24	3.33 e	64.33 c	77.00 b
48	3.66 e	76.33 b	89.33 a
72	4.00 e	78.33 b	92.00 a
Normal sprouts dry weight (mg)			
0	0.66 f	26.30 e	35.60 d
24	1.00 f	35.60 d	44.00 c
48	1.60 f	45.00 c	58.66 b
72	2.00 f	47.00 c	62.00 a
Vigor index (%)			
0	0.33 f	13.33 e	20.66 d
24	1.00 f	16.00 e	26.00 c
48	1.33 f	20.33 d	30.33 b
72	1.00 f	22.66 d	33.66 a
Growth rate (% etmal^{-1})			
0	0.13 i	8.98 h	11.93 f
24	0.33 i	11.00 g	15. 00 c
48	0.51 i	13.44 e	17.44 b
72	0.51 i	14.22 d	18.22 a

Note: The values followed by the same letter in each variable shows no significant difference based on DMRT α = 5%

seeds for 24 hours increased the viability and vigour when the panicles turned brown.

The seeds from green panicles showed the lowest viability and vigour even though they were dried for up to 72 hours. This is presumably because the seeds at this stage are not yet fully ripe, so the seeds cannot germinate even though they were dried until the moisture content was low. According to Surya (2008) the maturity level of seeds is an important factor that affects their ability to germinate and produce normally growing plants.

Seed Viability and Vigour in Relation to Position on Panicles

Seed position had a significant effect on viability and vigour for both genotype (Table 3 and 4). In "BOTOK 4" genotype, the seeds harvested from the base of the panicles showed higher viability and vigour. The seeds from the middle part of the panicle have low viability and vigor. It is assumed that the seeds in the middle part have undergone physiological maturity. while the seeds harvested from the tip position have low viability and vigour because they have not

Table 2. Effect of harvest criteria based on panicle color and drying time on seed viability and vigor of "BOTOK 10" genotypes

Drying time (hour)	Panicle colour		
	Green	Yellow 75%	Brown
Moisture content (%)			
0	14.11 a	13.22 b	13.11 b
24	12.55 b	11.66 c	11.55 cd
48	10.89 de	10.78 ef	9.22 g
72	10.11 f	9.11 g	8.88 g
Germination (%)			
0	0.66 h	47.66 g	67.33 d
24	1.00 h	53.00 f	78.66 c
48	1.66 h	62.00 e	84.66 b
72	2.00 h	70.66 d	90.00 a
Maximum growth potential (%)			
0	2.33 g	52.66 f	79.33 c
24	3.66 g	60.00 e	87.00 b
48	2.33 g	68.66 d	93.00 a
72	3.00 g	79.00 c	95.33 a
Normal sprouts dry weight (mg)			
0	0.66 i	30.33 h	49.00 d
24	1.00 i	33.66 g	52.66 c
48	1.66 i	37.66 f	56.33 b
72	2.00 i	45.00 e	59.66 a
Vigor index (%)			
0	0.66 f	13.00 e	16.00 cde
24	0.66 f	13.66 de	17.66 bc
48	0.67 f	15.66 cde	20.33 ab
72	1.00 f	17.33 bcd	21.66 a
Growth rate (%etmal^{-1})			
0	0.166 h	8.58 g	12.33 d
24	0.178 h	9.55 f	14.50 c
48	0.325 h	10.80 e	15.70 b
72	0.340 h	11.80 d	17.11 a

Note: The value followed by the same letter in each variable shows no significant difference based on DMRT α = 5%

reached physiological maturity. Martinatti et al. (2020) reported that seeds located at the tip of the panicles have lower weight and physiological performance.

The seeds of "BOTOK 10" genotype was different from "BOTOK 4" in that the seeds from the middle part of the panicles had the highest viability and vigor compared to the base and tip (Table 4). Martinatti et al. (2020) reported that millet seeds located in different regions of the panicles have different physical, physiological and other qualities, with the middle region having a high percentage of seed germination. Bernardi et al. (2019) also reported that the physiological quality of Sudan grass (*Sorghum sudanense* L.) seeds are dependent on seed size and density. The larger seeds have high germination rates and seed growth. Our results are in line with Komala et al. (2014). which states that seeds that are closer to the stalk will assimilate more molecules than seeds that are further from the stalk. Martinez et al. (2007) also stated that the distribution of assimilates starting from the base to the tip of the seeds would result in differences in the size of the seeds in each part.

Table 3. Effect of seed position on viability and vigor of “BOTOK 4” genotypes

Variable	Position		
	Base	Middle	Tip
		“BOTOK 4”	
Germination (%)	78.75 a	58.25 b	39.00 c
Maximum growth potential (%)	83.75 a	62.00 b	41.25 c
Vigor index (%)	56.50 a	38.00 b	23.00 c
Growth rate (%etmal^{-1})	19.63 a	13.98 b	8.57 c
Normal sprouts dry weight (g)	0.048 a	0.035 b	0.026 c

Note: The values on the same line followed by the same letter are not significantly different based on DMRT α = 5%

Table 4. Effect of seed position on viability and vigour of “BOTOK 10” genotypes

Variable	Position		
	Base	Middle	Tip
		“BOTOK 10”	
Germination (%)	80.00 b	91.50 a	76.25 b
Maximum growth potential (%)	88.50 b	97.25 a	84.75 c
Vigor index (%)	16.50 b	21.75 a	10.75 c
Growth rate (%etmal^{-1})	13.68 b	17.04 a	13.93 b
Normal sprouts dry weight (g)	0.050 b	0.061 a	0.053 ab

Note: Values on the same line followed by the same letter are not significantly different based on DMRT α = 5%

Conclusion

Foxtail millet seeds for both “BOTOK 4” and “BOTOK 10” genotypes had the highest quality when the panicles were brown (110-115 DAS) followed by drying for 72 hours. In these conditions “BOTOK 4” and “BOTOK 10” seed chlorophyll content reached its minimum level, seed moisture content was at 8.8%, and viability and vigor were at their highest. There are segmentation differences between “BOTOK 4” and “BOTOK 10” genotypes. High viability and vigor in “BOTOK 4” were recorded on seeds that were harvested from the base position of the panicle. On the other hand, “BOTOK 10” had high viability and vigor when the seeds were harvested from the middle position of the panicle.

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