BIODIVERSITAS
Volume 25, Number 11, November 2024
Pages: 4406-4414

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251140

Characteristics and quality of cashew (*Anacardium occidentale*) seeds across various agroecological conditions

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Manuscript received: 20 July 2024. Revision accepted: 22 November 2024.

Abstract. *Anugrah MH, Melati, Purwito A, Palupi ER. 2024. Characteristics and quality of cashew (*Anacardium occidentale*) seeds across various agroecological conditions. Biodiversitas 25: 4406-4415.* Cashew (*Anacardium occidentale* L.; Anacardiaceae) is a major plantation commodity developed mainly on marginal lands in Eastern Indonesia. This commodity is cultivated in various agroecological conditions, including wet, moderate, and dry climates. This research aims to provide information on the characteristics and quality of cashew seeds from different agroecological conditions and to explore the relationship between these conditions and seed storability. The research was conducted from November 2022 to December 2023. Seeds from two varieties were collected from seed orchards in various agroecological conditions. Seeds of Balakrisnan 02 variety were collected from a wet climate region (Bantul, Yogyakarta), and a moderate climate region (Sumbawa, West Nusa Tenggara). Seeds of Flotim variety were collected from a moderate climate region (Bangkalan, East Java), and a dry climate region (East Flores, East Nusa Tenggara). The seeds were stored at 16°C and 50% RH for six months. Physical, and physiological quality were observed during the storage. The result showed that drier agroecological conditions produce better physical quality seeds, such as larger seeds with thicker nut shells (fruit skin), and higher peroxidase activity and proline content. Planting the Balakrisnan 02 variety in moderate climate regions produces seeds with higher viability and vigor and longer storage capacity than those in wet climate regions. Meanwhile, Flotim, the drought-tolerant variety, when planted in the dry climate region produces seeds with higher viability but lower vigor and shorter storage capacity than those in moderate climate regions. Despite the differences in seed quality, planting the same variety in different agroecological conditions is still recommended to ensure seed availability due to different harvest times.

Keywords: Peroxidase activity, proline content, storage capacity, viability, vigor

INTRODUCTION

Cashew (*Anacardium occidentale* L., Anacardiaceae) trees are a vital commodity in plantations and have been extensively developed in Indonesia, particularly in the eastern regions. Witjaksono et al. (2023) reported that cashew nut production is distributed in marginal areas consisting of Nusa Tenggara and Southeast Sulawesi. According to Widiatmaka et al. (2015), there were several limiting factors of land suitability for cashew cultivation, including water availability, nutrient retention, available nutrients and rooting media. The International Nut and Dried Fruit Council Foundation (2021) reports that Indonesia is the world's 7th largest cashew nut producer after West Africa, India, Vietnam, East Africa, Cambodia, and Brazil. Cashew nut production in Indonesia in 2020-2021 reached 26,450 tons, accounting for 3% of total world production.

Several cashew varieties have been released, i.e. Balakrisnan 02 (B 02), Flotim, Imogiri, Segayung Muktiharjo 9 (SM 9), Meteor YK, Muna, PK 36, MR 851, and Asembagus II. The first two were the more largely planted. Balakrisnan 02 was an introduced variety from Kerala, India, and is more tolerant to helopeltis (mirids). Flotim is indigenous from East Flores and is a more drought-tolerant variety (Ministry of Agriculture 2015). Ferry (2012) reported that 14 cashew seed orchards have been established in various agroecological regions, mostly in Eastern Indonesia, to provide planting materials for propagation. Some varieties have been planted in more than one seed orchard. Therefore, it is interesting to evaluate the performance of the seed orchards in terms of the quality of the seeds they produce.

Agroecological conditions where the seed orchards are located significantly impact seed production (Rharrabti dan Sakar 2016; Botelho et al. 2019). Balogoun et al. (2016) demonstrated that the number of rainy days positively affects cashew production in the Central and North West Benin region, Republic of Benin (West Africa) which has a dry tropical climate. The normal monthly rainfall distribution in this area creates favorable conditions for the growth and formation of cashew nuts. An increase in production of 7-8% was correlated with higher rainfall between 1981-2010. However, in 2013-2014, the number of hermaphrodite flowers decreased due to the uneven rainfall distribution and temperature. Low rainfall (<80 mm per month) during the vegetative phase causes plants to lack water to start the flowering phase. On the other hand, high rainfall causes abortion during the fruit formation period. Ferrão et al. (2022) reported that the amount of monthly rainfall in Portugal affects hazelnut seed weight. Seed weight from the Viseu region (68 mm per month) was higher (1.70 g) than that of the Sernancelhe region (1.44 g) (56 mm per month). Widiatmaka et al. (2014) reported that production in major cashew production area of West Java, Yogyakarta, Central Java, West Nusa Tenggara, and East Nusa Tenggara was related to the availability of water in the field. Higher rainfall (over 2,247 mm per year) as well as low rainfall (below 987 mm per year) would decreased productivity.

Different agroecological conditions also affect the characteristics and quality of the seeds. Bello et al. (2019) reported that the cashew genotypes exhibited varied performance across various environments. The higher rainfall region (1100 to 1300 mm) in Benin produced a higher weight of nuts and apples than the lower one (900 to 1100 mm). Balogoun et al. (2016) reported that agroecological factors affect cashew flowering and fruiting, hence the production and quality of nuts. Environmental conditions such as rainfall, light intensity (including UV irradiation), soil type, and cultivation system (organic or non-organic) affect phytochemical compounds and metabolites of seeds (Bolling et al. 2011). Jaynaqov and Shavkat (2020) reported that peroxidase activity was higher in plants grown in drier areas, thus peroxidase activity can be used as an indicator of drought resistance genotypes. In addition, seed proline content was associated with the mechanism of plants to protect them from several abiotic stresses, such as water deficit and temperature changes (Miya and Modi 2015; Kijowska-Oberc et al. 2020).

Cashew seeds are orthodox with thick seed shells (Sruthi and Naidu 2023). However, germination decreases with a decline in seed moisture content during storage. One way of maintaining the quality of cashew seeds during storage is to keep the seed moisture content at 4-5% (Ministry of Agriculture 2015). Makale et al. (2020) reported that cashew seeds stored for over a year had poor quality, and could not be used as planting materials. Information regarding the character and quality of cashew seeds from various agroecological regions in Indonesia is essential for seed management, especially in determining appropriate handling to maintain the seed quality. This study investigates the physical, physiological, and chemical characteristics and the quality of cashew seeds from different agroecological conditions.

MATERIALS AND METHODS

The research was conducted from November 2022 to November 2023 at the Seed Storage and Quality Testing (SSQT) Laboratory, Reproductive Biology and Biophysics Laboratory, Microtechnic Laboratory, and Cikabayan Experimental Station, Department of Agronomy and Horticulture, IPB University, Bogor, West Java, Indonesia.

Seed source

Cashew seeds from two varieties were collected from Seed Orchards (SO) in various agroecological conditions. Seeds of the Balakrisnan 02 variety were collected from a wet climate region (Bantul, Yogyakarta), and a moderate climate region (Sumbawa, West Nusa Tenggara). Seeds of the Flotim variety were collected from a moderate climate region (Bangkalan, East Java), and a dry climate region (East Flores, East Nusa Tenggara). Seeds were harvested by farmers that have been trained as seed producers. Ripe fruits were harvested, and the nuts (seeds) were separated from the apples. The seeds were dried for 3-4 hours at 30 - 33°C, then packed in thick cardboard covered with styrofoam and sent to the SSQT Laboratory. The seeds were then put in propylene (PP) plastic bags and stored in the room at 8°C and 50% RH.

Agroecological conditions of the SO were obtained from data provided by the Meteorology, Climatology, and Geophysics Agency (*BMKG*) over the past 10 years. The climate type of each SO was determined using the Schmidt and Ferguson (1952) classification. Agroecological classification is based on the Q value as the average ratio of dry months to wet months. Months with rainfall <60 mm are classified as dry, while months with rainfall >100 mm are classified as wet. Based on these criteria, the Bantul falls into the wet climate region (0<Q<0.6), Bangkalan and Sumbawa moderate climate regions (0.6<Q<1), and East Flores a dry climate region (1<Q<7) (Table 1).

Table 1. Agroecological conditions of the cashew nut seed orchards

Agroecological conditions	Balakrisnan 02 variety		Flotim variety	
	Bantul	Sumbawa	Bangkalan	East Flores
Altitude (masl)	50-100	400-500	2-10	200-400
Temperature (°C)	25.9-26.5 ($\bar{x}$=26.2)	25.9-26.8 ($\bar{x}$=26.4)	28.7-29.3 ($\bar{x}$=28.9)	27.6-28.8 ($\bar{x}$=28.1)
Air relative humidity (%)	80.9-84.4 ($\bar{x}$=82.6)	82.3 - 87.0 ($\bar{x}$=83.9)	72.2-77.9 ($\bar{x}$=75.7)	74.5-78.9 ($\bar{x}$=76.4)
Rainfall (mm per year)	1264.9-3001.4 ($\bar{x}$=2266.5)	162.2-2391.4 ($\bar{x}$=1519.4)	1172.3-2314.3 ($\bar{x}$=1179.0)	376.3-2407.7 ($\bar{x}$=1179.0)
Wet months per year	7	6	6	4
Dry months per year	4	5	5	6
Light intensity (h per day)	5.82	6.93	6.35	6.31
Soil type	Cambisols	Mediteran	Laterite	Mediteran
Soil pH	4.5±1	7±0.5	7±0.5	7±0.5
Location	Karang Tengah, Kepanewon, Bantul, Yogyakarta	Labuhan Badas, Labuhan Badas, Sumbawa, West Nusa Tenggara	Tanjung Bumi, Tanjung Bumi, Bangkalan, East Java	Balukhering, Lewolema, East Flores, East Nusa Tenggara
Coordinates	7.940955S; 110.394E	8.476924S; 117.36403E	6.900649S; 113.061442E	8.208891S; 122.929629E

Note: Processed from BMKG data for the last 10 years (2013-2022) (www.https://dataonline.bmkg.go.id), BPS reports for each district

Characteristics and quality of cashew seeds

Physical characteristics of cashew seeds

Differences in the physical characteristics of seeds of the same variety but from different agroecological conditions were assessed based on the following criteria: (i) Weight of 1000 seeds: determined according to the ISTA method (2018) by weighing 100 pure seeds with eight replications, and calculating the Coefficient of Variation (CV). The weight of 1000 seeds are calculated as the average weight multiplied by 10 if the CV value does not exceed 4%. (ii) Seed length and width: The length and width of 10 seeds from each SO were measured using a calliper with an accuracy of 0.01 mm (Hu et al. 2018) and repeated four times. (iii) Seed dry weight: 4 seeds from each SO, individually weighed and sliced to a thickness of less than 7 mm, then dried in an oven at 105±2°C for 17 hours (Isa and Aderotoye 2018).

Nutshell and seed coat thickness: the thickness of the cashew nutshell of 10 seeds from each SO was measured using a caliper with an accuracy of 0.01 mm. The seed coat (testa) was measured using a microscope with 4x magnification.

Physiological quality of cashew seeds

The physiological quality of seeds from different agroecological conditions was assessed based on the following criteria: (i) Germination Percentage (GP): Calculated as the percentage of normal seedlings on the first count (17 days after sowing DAS^{-1}) and final count (30 DAS) (ISTA 2018). (ii) Maximum Germination Capacity (MGC): calculated as the percentage of germinated seeds (both normal and abnormal seedlings) in the final count (Sadjad et al. 1974). (iii) Seedling Growth Rate (SGR): Calculated from normal seedlings' dry weight divided by the number of normal seedlings on 25 DAS (Copeland dan McDonald 1985). (iv) Vigor Index (VI): Calculated as the percentage of normal seedlings on the first count (17 DAS) (ISTA 2018). (v) Germination Speed (GS): Calculated as the percentage of normal seedlings appearing each day from the first to the final count (Sadjad 1994). (vi) Electrical Conductivity (EC): Calculated as the electrical conductance of soaking water of 20 g seeds for 24 hours in ddH_2O (double distilled water) (ISTA 2018).

Chemical quality of cashew seeds

The chemical characteristics of cashew seeds from the four SOs were assessed based on the following criteria: (i) The peroxidase enzyme activity was analyzed according to Singh et al. (2015) using the embryonic axis for samples. (ii) Proline compounds were analyzed according to Kijowska-Oberc et al. (2020) using 1 g embryo for samples.

Data analysis

Data were analyzed using the T-test (Student's T-test) at a confidence interval of 95%. Correlation analysis of the chemical character and physiological quality of seeds was carried out in Excel 2016 software.

RESULTS AND DISCUSSION

Physical characteristics of cashew seeds

The physical characteristics of the seeds of Balakrisnan 02 variety from Sumbawa SO (1000-grain weight, seed length, dry weight, and nutshell thickness) are higher than those of the seeds from Bantul SO, except for the width and thickness of the seed coat (Table 2). Seeds of Flotim variety from East Flores SO have physical characteristics (1000-grain weight, seed length, seed width, seed dry weight, and fruit skin thickness) that are higher than seeds from Bangkalan SO, except for the value of seed coat thickness. These data showed that both Balakrisnan 02 and Flotim varieties have better physical characteristics when planted in drier areas, as long as they are within their adaptation range.

The rainfall at the East Flores SO is lower (1179.0 mm per year) with a higher number of dry months per year (6 months) than at the Bangkalan SO (1699.5 mm per year; and 5 dry months). A similar situation occurs between the Sumbawa and Bantul seed orchards. Drier conditions are thought to increase the accumulation of food reserves during seed development because the rate of photosynthesis is higher, leading to more assimilates being translocated to the developing fruit/seed compared to wetter conditions. Yin et al. (2020) explained that seed-filling is a dynamic process that determines the seed size and metabolite content. Their research proved that corn seeds originating from geographic areas with higher seed filling rates (0.55 g per day) produced higher seed dry weights than from areas with lower seed filling rates (0.40 g per day).

Table 2. Physical characteristics of cashew seeds from four SOs

Variety	Seed orchards	Weight of 1000 seed (g)	Length of nut (mm)	Width of nut (mm)	Dry weight per nut (g)	Thickness of nutshell (mm)	Thickness of seed coat (μm)
Balakrisnan 02	Bantul	6151.8^b	30.27^b	18.93^a	1.67^b	2.42^b	99.69^a
	Sumbawa	6244.2^b	31.06^a	16.79^b	1.85^a	3.18^a	51.82^b
	Var description	-	29.80	17.80	-	-	-
Flotim	Bangkalan	5895.8^b	30.4^b	12.87^b	1.95^b	3.24^b	80.72^a
	East Flores	8634.8^a	36.6^a	20.83^a	2.24^a	3.46^a	27.20^b
	Var description	-	30.0-49.0	11.0-32.0	-	-	-

Note: The number for each variable followed by a different letter shows a significant difference based on the results of the T-test (Student's T-test) at a 95% confidence interval

Agroecological conditions influence the phenology of flowering and fruiting in cashew plants. Flowering begins with the emergence of flower buds. In Bantul flower buds emerge around April, and harvesting takes place in August-September, earlier than in Sumbawa where flowering starts around August-September and harvesting in December-January (Table 3). A similar trend with different phenological changes was observed in the Flotim variety, where harvesting in East Flores occurs earlier than Bangkalan - planting the Balakrisnan 02 variety in drier areas delays flowering. In contrast, the Flotim variety tends to accelerate flowering. Although the main harvest period has ended in Bantul, Bangkalan, and East Flores seed orchards, flowering continues at a low intensity, so seeds are still available in the months following the harvest. The variations in flowering and harvest times in different agroecological conditions can aid in planning seed procurement for better availability. However, changes in flowering and fruiting phenology under different agroecological conditions should be studied over a longer period before being incorporated into seed procurement planning. In particular, the flowering pattern in Sumbawa, which starts during the dry months and harvests during the wet months, requires detailed observation. Harvesting seeds in the wet months carries the risk of reduced quality due to higher water content. Oza and Solanki (2021) reported that high rainfall or humidity during the flowering and harvest affected the quantity and quality of fruit/nuts. In addition, there was also an increase in pest and disease infestations by pathogens. According to Parlindo et al. (2022), the fruiting bodies of the pathogenic fungus *Rigidoporus* sp. overgrew in the rainy season of Eastern Bali. They attached to the stem of the infected tree and the tree trump, and difficult to control, thus became a significant disease of cashew (Parlindo et al. 2023). This condition was detrimental to cashew farmers because the flowers on productive trees would fall quickly.

The period from the appearance of flower buds to harvest for the Balakrisnan 02 variety spans 6 months in wet (Bantul) and moderate (Sumbawa) climates. Meanwhile, the Flotim variety takes 5 months from the appearance of flower buds to harvest in moderate climates (Bangkalan) and 7 months in dry climates (East Flores).

The nutshell is brown with varying shades among the seed orchards. The nutshell of Balakrisnan 02 has a darker shade of brown when grown in the drier area (Sumbawa) compared to the wet area (Bantul). In contrast, the Flotim variety has a lighter shade of brown when grown in the drier area (East Flores) compared to the moderate area (Bangkalan). The kidney-shaped cashew seeds are consistent across the four seed orchards, with the proximal part (point of attachment to the pseudo-fruit) larger than the distal part (Figure 1).

The nutshell is thick and hard, protecting the embryo inside. The embryo is large, filling the seed cavity, with the cotyledons being the major part and the embryo axis at the proximal end (Figure 2). The hilum is located at the curve of the kidney-shaped seed. The cotyledons have slightly shrunk after the seed was stored for six months.

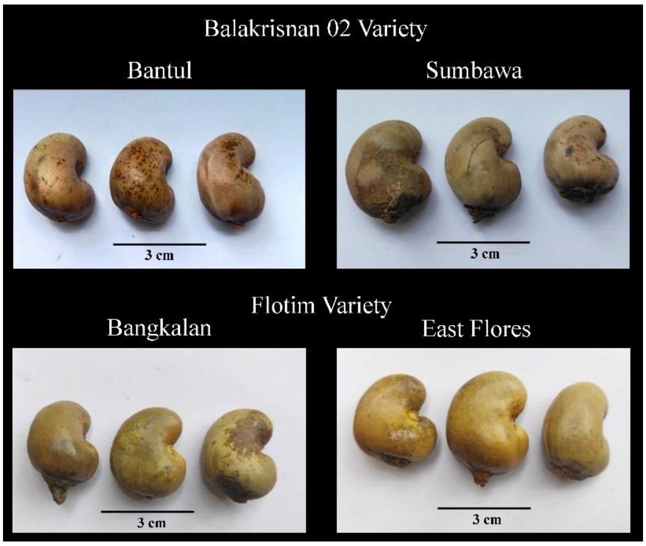

Figure 1. Shape, size, and nutshell color of cashew seeds from four seed orchards

Table 3. Rainfall intensity, flowering period, and seed harvest in 2022

Seed orchards	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Duration (mo.)
Bantul													6
Sumbawa													6
Bangkalan													5
East Flores													7

Legend:
- Period of flower bud emergence
- Period of flower blooming (anthesis)
- Period of fruit development
- Period fruit maturation and harvest
- Wet months
- Moderate (humid) months
- Dry months

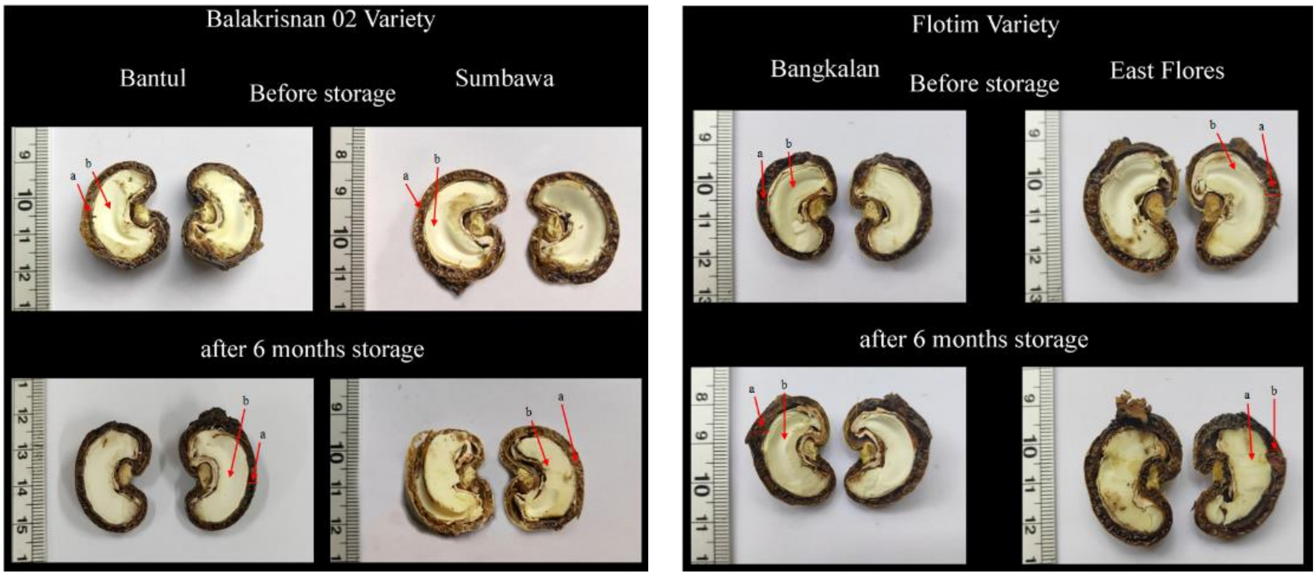

Figure 2. The internal structure of cashew seeds from four seed orchards (a = nutshell, b = cotyledons)

Table 4. Changes in seed viability and vigor of Balakrisnan 02 variety during storage

SO	Seed storage (month after storage)						
	0	**1**	**2**	**3**	**4**	**5**	**6**
				Germination (%)			
Bantul	84.0^b	81.7^a	78.3^a	69.5^a	59.7^b	53.8^b	51.5^b
Sumbawa	85.0^a	81.5^a	77.0^b	67.5^b	64.8^a	57.8^a	70.8^a
				Maximum germination capacity (%)			
Bantul	86.7^a	86.7^a	85.7^a	79.5^a	73.5^a	67.5^b	67.5^b
Sumbawa	86.8^a	87.7^a	82.7^b	75.5^b	72.5^a	71.5^a	80.5^a
				Seedling growth rate (g per seedling)			
Bantul	1.14^a	1.06^a	0.94^a	1.37^a	1.12^a	0.85^b	0.84^b
Sumbawa	1.15^a	1.07^a	0.94^a	1.18^b	1.08^b	1.06^a	0.88^a
				Moisture content (%)			
Bantul	9.96^a	9.69^a	8.98^a	8.6b^a	8.40^a	8.70^a	7.72^a
Sumbawa	8.14^b	6.76^b	6.47^b	6.33^b	6.17^b	6.14^b	6.24^b
				Vigor index (%)			
Bantul	36.3^a	24.3^b	20.3^b	16.3^b	20.3^b	15.7^b	14.3^b
Sumbawa	35.3^b	31.7^a	26.3^a	28.3^a	21.7^a	22.3^a	20.3^a
				Germination speed (% normal seedling per day)			
Bantul	4.49^b	4.07^b	3.86^a	3.60^b	3.10^b	2.80^b	2.91^b
Sumbawa	4.56^a	4.16^a	3.76^b	3.71^a	3.27^a	3.18^a	3.79^a
				Electrical conductivity (μS cm^{-1} g^{-1})			
Bantul	1.67^b	2.51^b	3.02^b	5.11^b	4.95^b	6.61^b	8.27^b
Sumbawa	7.77^a	10.15^a	14.77^a	16.77^a	18.91^a	18.81^a	19.15^a

Note: The number for each variable during the nth storage month followed by a different letter shows a significant difference based on the results of the T-test (Student's T-test) at a 95% confidence interval

Physiological quality of cashew seeds

In general, cashew seeds of the Balakrisnan 02 variety from Sumbawa exhibit higher initial quality compared to seeds from Bantul, as indicated by the pre-storage seed quality test. After 6 months of storage, seeds from Sumbawa maintained higher viability (GP, MGC, and SGR) and vigor (VI and GS) than seeds from Bantul (Table 4). This suggests a slower decline in seed quality for Sumbawa (16% reduction in GP) compared to Bantul (38% reduction in GP) which could be due to differences in water content are thought to be one of the causes of differences in the rate of decline in seed quality. Seeds from Sumbawa experienced a greater decrease in water content than seeds from Bantul (Table 4). The thinner seed coats of seeds from Sumbawa (51.82 µm) compared to seeds from Bantul (99.69 µm) (Table 2) could potentially explain the higher rate of loss in water content.

The electrical conductivity indicates the presence of metabolites contained in the seed-soaking water due to cell membrane lysis indicating a decrease in membrane integrity (Phartyal et al. 2003). The higher the EC suggests more metabolites in the soaking water, indicating the higher level of cell membrane leakage, or the higher level of seed deterioration. Throughout the storage period, the EC of seeds from Bantul is consistently lower than seeds from Sumbawa, which implies that seeds from Sumbawa (with a thicker nutshell) have experienced a higher deterioration than seeds from Bantul (thinner nutshell), contrary to the viability and vigor data. The high-value metabolite leakage in seeds from Sumbawa is thought to come from the leakage of the nutshell cells which is much thicker than the seeds from Bantul. Arif et al. (2020) reported that the variation in EC of oil palm seeds could be attributed to the thick shell structure, with metabolites arising from the deterioration of this shell. Further detailed observations on the cell membrane damage are necessary to clarify this phenomenon.

The seed quality of Flotim variety from Bangkalan was higher and declined slower compared to seeds from East Flores as indicated by viability (GP, MGC, SGR) and vigor (VI and GS) during the storage period (Table 5). Similar to the Balakrisnan 02 variety, seeds from the Flotim variety showed that seed lots with the lower decline of quality had lower water content; in this case, seeds from Bangkalan had lower water content than seeds from East Flores (Table 5). Bangkalan is located at lower elevation (2-10 masl) than East Flores (200-400 masl). Apart from the altitude, the difference in agroecological components between the two seed orchards is that Bangkalan has experienced higher average rainfall and more wet months over the past decade compared to East Flores. On the other hand, East Flores experiences long dry months, during which the onset of flowering, fruit development, and seed maturation mostly occur, potentially affecting the quality of seeds produced (Table 1). Maity et al. (2023) reported that increasing temperatures negatively impact seed filling speed, duration, and timing, resulting in lower seed weight. However, our study indicates a different trend: Flotim, the drought-tolerant variety, has a better physiological quality when planted in moderate climate areas than those from dry areas.

The higher viability and vigor of seeds from Bangkalan than those from East Flores are followed by their EC (electrical c onductivity) values. The thicker seed coat of the Bangkalan seeds (80.72 µm vs. 27.20 µm) likely contributed to the slower decline in seed quality. However, the observation that seed lots with higher viability and vigor also showed higher EC values, as seen in the Balakrishnan variety, could not be explained in this study.

Chemical quality of cashew seeds

The analysis revealed that the peroxidase activity of seeds of the Balakrisnan 02 variety from Sumbawa (0.176 UA per g) was not significantly different from that of Bantul (0.110 UA per gram). In contrast, seeds of the Flotim variety from East Flores had higher peroxidase activity (0.330 UA per g) than seeds from Bangkalan (0.298 UA per g) (Figure 3). According to Babaei and Ghanbari (2016), an increase in peroxidase activity is an initial response to mild stress induced by prolonged light intensity. These findings suggest that the Flotim variety in East Flores, which experiences a dry climate, undergoes mild stress and therefore shows higher peroxidase activity compared to seeds from Bangkalan. In addition, the peroxidase activity of all seed lots tended to increase during storage. The increase indicates the activation of seed defense mechanisms against unfavorable conditions, such as the production of free radicals that contribute to seed deterioration (Mithun et al. 2023).

Table 5. Changes in seed viability and vigor of Flotim variety during storage

SO	Seed storage (month of storage)						
	0	1	2	3	4	5	6
				Germination (%)			
Bangkalan	72.0^b	60.5^b	60.7^a	52.7^a	55.5^a	61.5^a	65.7^a
East Flores	77.3^a	70.0^a	55.8^b	53.5^a	47.7^b	50.5^b	51.8^b
				Maximum germination capacity (%)			
Bangkalan	90.7^b	77.8^b	85.7^a	76.5^a	77.5^a	80.5^a	80.5^a
East Flores	93.7^a	92.8^a	86.8^a	71.7^b	68.2^b	66.2^b	57.5^b
				Seedling growth rate (g per seedling)			
Bangkalan	1.16^a	1.00^b	1.27^a	1.17^a	1.00^b	1.00^a	0.92^a
East Flores	1.17^a	1.26^a	1.07^b	0.90^b	1.03^a	1.00^a	0.86^b
				Moisture content (%)			
Bangkalan	7.69^b	7.49^a	6.49^b	6.44^b	6.19^b	6.13^b	5.93^b
East Flores	8.25^a	7.50^a	7.40^a	7.42^a	7.28^a	7.19^a	6.98^a
				Vigor index (%)			
Bangkalan	41.0^a	31.7^a	25.7^a	20.7^a	21.7^a	22.3^a	18.3^a
East Flores	31.0^b	24.3^b	19.7^b	14.3^b	13.7^b	15.7^b	13.7^b
				Germination speed (% normal seedlings per day)			
Bangkalan	3.98^a	3.17^a	3.00^a	2.54^a	2.98^a	3.36^a	3.40^a
East Flores	3.76^b	3.37^a	2.69^b	2.45^b	2.45^b	2.47^b	2.51^b
				Electrical conductivity (µS cm^{-1} g^{-1})			
Bangkalan	5.56^a	11.98^a	11.01^a	11.58^a	12.88^a	12.72^a	12.34^a
East Flores	4.80^b	7.36^b	6.36^b	7.87^b	8.13^b	8.81^b	9.78^b

Note: The number for each variable during the n^{th} storage month followed by a different letter shows a significant difference based on the results of the T-test (Student's T-test) at a 95% confidence interval

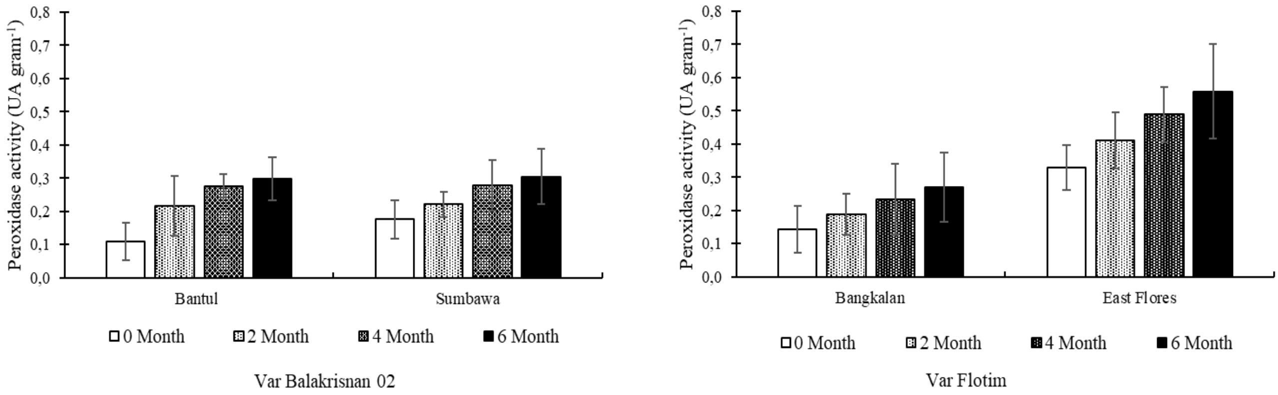

Figure 3. Peroxidase activity of cashew seeds of Balakrisnan 02 and Flotim varieties during storage

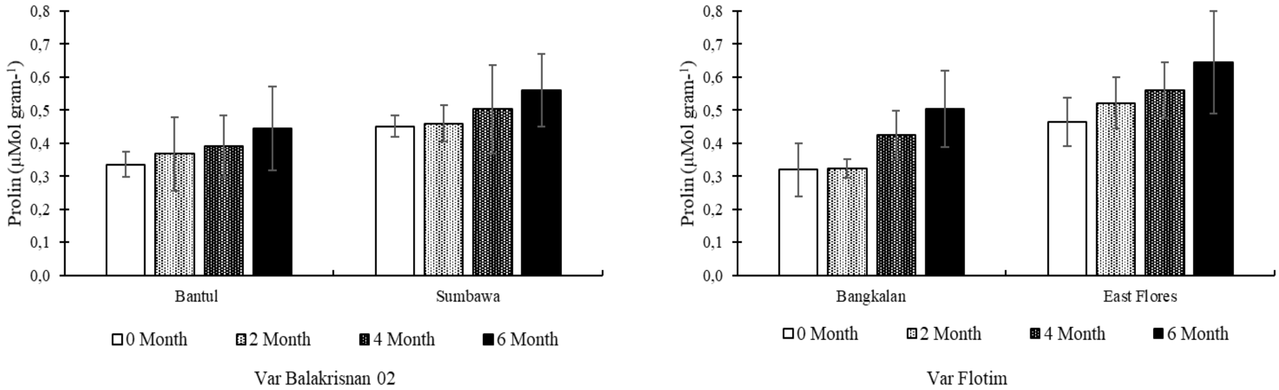

Figure 4. Proline content of cashew seeds of Balakrisnan 02 and Flotim varieties during storage

The seeds' proline content is influenced by the environmental conditions during the growth of the parental plant. Proline content increases under water stress conditions (Masheva et al. 2022). Seeds of the Balakrisnan 02 variety from Sumbawa showed higher proline content (0.451 µMol g^{-1}) than seeds from Bantul (0.336 µMol g^{-1}), reflecting the drier agroecological conditions in Sumbawa. Similarly, in Flotim variety, the proline content in seeds from East Flores (0.464 µMol g^{-1}) was higher than in seeds from Bangkalan (0.320 µMol g^{-1}) (Figure 4). A similar phenomenon was also reported by Kijowska-Oberc et al. (2023) in seeds of Acer, where an increase in environmental temperature led to higher proline content. Proline compounds regulate osmotic pressure during drought conditions, thereby reducing cell membrane damage. Moreover, proline also protects lipid structures within cell membranes from damage by free radicals (such as H_2O_2) that oxidize membrane components.

The proline content in the seeds of both cashew varieties tends to increase during storage, albeit at different rates. This aligns with the finding of Kong et al. (2015) who reported an increase in proline compounds in oat seeds (*Avena sativa* L.) during 12 months of storage at temperatures <10°C. According to Miya and Modi (2015), proline acts as an osmoprotectant that stops reactive oxygen species and enhances antioxidants, thereby slowing down the deterioration process. The slower quality decline of seeds from Sumbawa is also thought to be influenced by the higher proline content than seeds from Bantul. However, the high proline content of seeds from East Flores was accompanied by a faster decline in seed physiological quality compared to seeds from Bangkalan which has lower proline content. This phenomenon remains unclarified in this study.

Correlation of chemical characteristics and physiological seed quality

Peroxidase activity and proline content of the Balakrisnan 02 variety were negatively correlated with MC (r = -0.64 and r = -0.73 respectively) but positively correlated with EC (r =0 .53 and r = 0.88 respectively). This suggests that an increase in the seeds' moisture content will reduce peroxidase activity and proline content, and conversely, an increase in peroxidase activity and proline content enhances EC. At high moisture content, seeds do not experience stress conditions, thereby preventing the formation of peroxidase as an antioxidant and proline as an osmoregulatory substance. According to Gulen and Eris (2004), peroxidase enzyme accumulation occurs when plant cells experience stress conditions in response to stress factors. Increased peroxidase activity prevents oxidative stress while increasing proline content protects cells from damage due to osmotic pressure, both of which inhibit seed deterioration. Vijayalakshmi et al. (2018) reported a negative correlation (r = -0.996) between maize seed peroxidase values and EC. Padilha et al. (2022) also reported no correlation between increased proline and seed vigor. However, in this study, increased peroxidase activity and proline content were accompanied by an increased electrical conductivity. Further detailed research is needed to clarify this phenomenon.

Discussion

Based on the description, the Balakrisnan 02 variety is recommended for planting in wet to moderate climate regions. Therefore, the seed orchards were built in Bantul, which falls into the wet climate category, and Sumbawa, which falls into the moderate climate category. Apart from climatic conditions, the two seed orchards are located at different altitudes, have different soil types, and soil pH. Meanwhile, the Flotim variety is recommended for planting in moderate (Bangkalan SO) and dry climates (East Flores SO) with a rainfall adaptation range of 1179.0-1699.5 mm per year, both having different soil types and altitudes.

Seeds of the Balakrisnan 02 and Flotim varieties from drier areas exhibit better physical characteristics than those from wetter climate areas. Gomes et al. (2023) reported that *Anadenanthera colubrina* (Vell.) Brenan seeds collected in 2016, under low rainfall conditions (270 mm/year), had a higher 100-seed weight (14.74 g) compared to seeds collected in 2017, under higher rainfall (391 mm/year). The finding aligns with our study, which shows that drier conditions positively impact the physical characteristics of seeds.

Based on viability and vigor, seeds of the Balakrisnan 02 variety from Sumbawa, which is located at a higher altitude (400-500 masl) have better physiological quality compared to seeds from Bantul (50-100 masl). The influence of altitude on the performance and quality of seed was also reported by Yucedag et al. (2021), in which Juniper (*Juniperus drupacea* (Labill.) seeds from higher elevation areas (1400 masl) had the highest germination (53.5%±2%) compared to elevations of 1000 and 1200 masl. Other agroecological factors may also play a role in the production of higher-quality seeds. The rainfall in Sumbawa is lower (1519.4 mm year^{-1}) with higher light intensity (6.93 hours per day) compared to Bantul with rainfall of >2000 mm year^{-1} and light intensity of 5.82 hours day^{-1}. Moreover, Bantul does not experience pronounced dry months, so the harvesting was carried out during periods of moderate rainfall. Abel and Boelt (2017) reported that increasing rainfall reduced the seed set of *Lolium perenne* L. by 60%, leading to lower seed production. Increased precipitation raises humidity, resulting in a diminished seed set. High canopy humidity can affect two critical factors: pollen longevity and stigma receptivity. Excessive humidity can cause pollen grains to swell excessively, disrupting their interaction with the stigma and making them defective. It also reduces the stigma's receptivity period, shortening the time available for pollination and the progamic phase (Shivanna and Heslop-Harrison 1981).

Response to agroecological conditions varied between varieties. The physical quality of both varieties is improved when planted in drier areas. However, the physiological quality of Balakrisnan 02 seeds is higher in drier areas, whereas the Flotim variety shows an increase in physiological quality under opposite (less dry) conditions. The Balakrisnan 02 variety originates from Kerala, India, which has a wet climate. Its distribution is typically suited to wet to moderate climates. In contrast, the Flotim variety originated from the Imogiri population in Yogyakarta, adapted well to East Flores, and was released as a local variety in East Flores (Ferry 2012). Observations in this study revealed that seeds of Flotim variety the from Bangkalan (a moderate climate area) have higher physiological quality compared to seeds from East Flores since the agroecological condition of Bangkalan is closer to the region where its initial population originated (Imogiri).

Chemical analysis of the two varieties also showed that seeds from drier areas performed better under storage conditions. For peroxidase activity, seeds of the Balakrisnan 02 variety from Sumbawa (moderate climate) had higher values than those from Bantul (wet climate). Similarly, seeds of the Flotim variety from East Flores (dry climate) exhibited higher peroxidase activity than those from Bangkalan (moderate climate). Regarding proline content, seeds of the Balakrisnan 02 variety from Sumbawa (moderate climate) had higher proline levels than those from Bantul (wet climate). In the Flotim variety, seeds from East Flores (dry climate) had higher proline content than those from Bangkalan (moderate climate).

To conclude, drier agroecological conditions produce cashew seeds with better physical quality, such as larger seeds with thicker nutshell, and higher peroxidase activity and proline content. Planting the Balakrisnan 02 variety in moderate climate regions produces seeds with higher viability and vigor, and maintains viability for a longer periods than those planted in wet climate regions. In contrast, the Flotim variety planted in dry climate regions produces seeds with higher viability but lower vigor and shorter storability compared to those planted in moderate climates regions. Despite these differences in seed quality, planting the same variety in different agroecological conditions is still recommended to ensure seed availability due to varying harvest times.

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