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Physiological Quality Testing of Local Chili Seeds of *Capsicum frutescens* Species

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Abstract

The high viability of cayenne pepper seeds is a critical factor influencing plant production levels. Research on the quality of chili seeds is essential to determine the physiological characteristics of seeds across various chili varieties. This study aims to provide information regarding the viability and vigor of *C. frutescens* chili seeds. The research was conducted from February to April 2024 at the Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University. A Randomized Complete Block Design (RCBD) was employed, focusing on cayenne pepper varieties with four replications. The results of the observations indicated that the highest 1000-seed weight was recorded for the *C. frutescens* Feira variety (5.33 g). In comparison, the lowest weight was observed in the Cakra Putih variety (4.08 g). The DHL test results indicated that the Harita variety exhibited the lowest potential for leakage, measuring at 173.80. Additionally, the Harita variety demonstrated the highest vigor values in the Vigor Index, Growth Rate, Growth Simultaneity, and Sprout Growth Rate. The results of the Pearson Correlation Analysis revealed a significant positive relationship between Radicle Emergence (RE) and Dry Biomass (DB), with a correlation coefficient of 0.85. Estimating seed vigor can be effectively accomplished through the Radicle Emergence (RE) test, which is easier and faster.

Keywords: Cayenne Pepper, Correlation, Germination, Viability, Vigor

1. Introduction

Chili pepper (*Capsicum frutescens*) is a member of the Solanaceae family and is widely cultivated in Indonesia. The fruit of the chili pepper is commonly used in processed food products, medicinal applications, and as a cooking spice. Chili peppers contain antioxidant compounds, including capsaicin, carotenoids, violaxanthin, and lutein. The plant exhibits morphological characteristics such as a crown color that ranges from whitish green to greenish yellow, and fruit colors that can be green, white, or greenish white. The leaves are typically deltoid in shape (Undang et al., 2015). The plant can reach a height of up to 2 meters and has a growth habit that is either herbaceous or semi-shrubby. Chili pepper plants usually produce two or more flowers per node and can live for 2 to 3 years. *Capsicum frutescens* L. is a lowland plant that thrives in various growing media, including soil, cocopeat, and vermicompost (Bijeta et al., 2018).

Chan et al. (2021) classify seed quality into three types: physical, genetic, and physiological. Physical quality is related to the physical properties of seeds, such as weight, size, shape, integrity, and seed condition due to pest and disease attacks or mechanical damage. Genetic quality is the quality of seeds related to the characteristics passed down from parent plants to their offspring. Physiological quality is the quality of seeds associated with the physiological properties of seeds such as viability, vigor, germination power, and seed storage capacity. Suppose the seed quality does not comply with the Indonesian Ministry of Agriculture standards. The seeds will not pass the certification process or receive a seed label in that case. Seeds sold without labels are categorized as illegal seeds. Seed viability is defined as the ability of seeds to grow in optimum conditions, while seed vigor is defined as the ability of seeds to grow in sub-optimal conditions. Seed viability and vigor are parameters determined mainly by the physiological quality of the seeds. Physiological quality will be maximized when it reaches physiological

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maturity and will experience deterioration during storage (Ratnaningtyas & Pudjihartati, 2019). Azmi & Rahayu (2022) conducted a study on the effect of soaking time on chili seed germination, using the Germination Power test. This study used a more practical test, the Radicle Emergence Test, which is closely positively correlated with the Germination Power variable. This study aims to test the viability and vigor of seeds from four varieties of cayenne pepper.

2. Material and Methods

This research was conducted from February to April 2024 at the Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University (6°33'33.4"S 106°43'49.0"E) at an altitude of 250 meters above sea level. The tools used in this study were: electric germinator, oven, plastic box, digital scale, microscope, and conductivity meter, while the materials used were chili seeds, distilled water, and tetrazolium solution. This study used a Randomized Complete Group Design (RKLT) with one factor, namely the chili variety *C. frutescens* (Bonita, Cakra Putih, Feira, Harita), with four replications.

Seed harvesting is carried out in the greenhouse on chili fruit that has physiologically matured based on the change in color of the ripe chili fruit, which is fully red (Kusumawardana et al., 2019), and when the plant is 38-44 days old after anthesis. (Suharsi et al., 2015) and continued with seed extraction. The research flow diagram describes the entire research procedure from the initial to the final stage of concluding the research results (Figure 1).

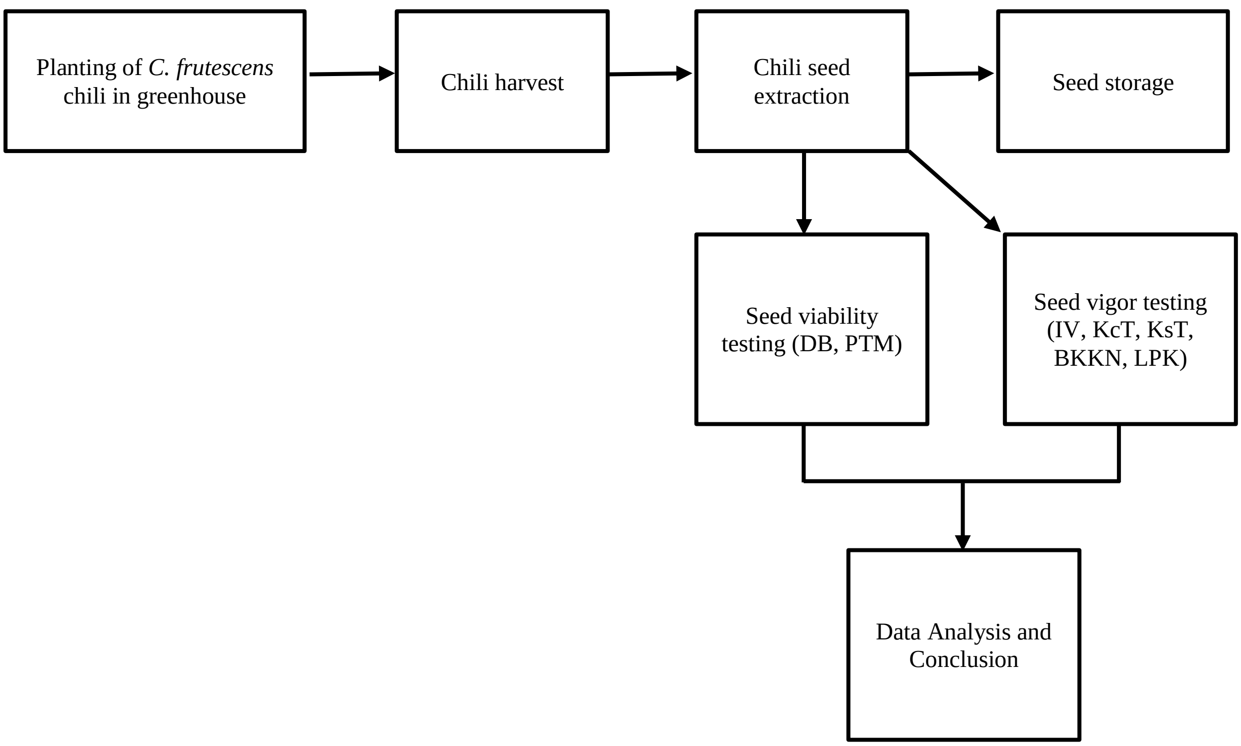

Figure 1. *C. frutescens* chili seed research

Determining the weight of 1000 seeds is calculated from data of 100 seeds by first calculating the variance, standard deviation, and coefficient of variance using the following formula (ISTA, 2018):

$$S^2 = \frac{N \sum X^2 - (\sum X)^2}{N(N-1)}$$

Seed Viability and Vigor Testing

Viability and vigor testing of chili seeds is carried out using standard test methods based on guidelines ISTA (2018) that include:

2.1. Standard test for viability and vigor testing

Seed viability and vigor testing based on the Top (ISTA, 2018) Paper Test method, using 50 seeds for each replication, with four replications for each treatment. The seeds were germinated in a plastic box, the germination media used were CD paper and two sheets of tissue paper moistened with distilled water, germinated in an electric germinator at a temperature of 20-30 °C. The variables observed were:

2.1.1. Germination Power (%)

The percentage of Germination Power (DB) is calculated based on the ratio between the number of normal sprouts 1 and 2 and the total seeds planted. The first and second observations were on the 7th and 14th days (ISTA 2018). Germination power is calculated using the formula:

$$\%DB = \frac{\Sigma(KN\ Count\ 1 + KN\ Count\ 2)}{\Sigma\ Planted\ seeds}\ x\ 100\%$$

2.1.2. Maximum Growth Potential (%)

Maximum growth potential (PTM) is calculated based on the number of sprouts that grow normally and abnormally until the end of the observation period (Sadjad, 1993). Maximum growth potential is calculated using the formula:

$$PTM = \frac{\Sigma\text{Growing Seeds until the end of observation}}{\Sigma\, Planted\ seeds}\ x\ 100\%$$

2.1.3. Vigor Index (%)

Observation of the vigor index (IV) was carried out on the number of normal sprouts (KN) during the first germination power count (Copeland & McDonald, 2001), namely on the 7th day. The vigor index is calculated using the formula:

$$IV = \frac{\Sigma(KN\ Count\ 1)}{\Sigma\, Planted\ seeds}\ x\ 100\%$$

2.1.4. Growth rate (%KN/etmal)

The growth rate (KCT) is measured based on the addition of the number of sprouts every day or etmal (24 hours) during the germination test period (Sadjad, 1993), namely day 1 to day 14. The formula for calculating the growth rate is written as follows:

$$K_{CT} = \Sigma_{i=1}^{14} \frac{\%\text{ Normal sprouts of} - \text{i}}{\text{Observation hour in} - \text{i}/24}\ x\ 100\%$$

2.1.5. Growth simultaneity (%)

The simultaneity of growth (KST) is calculated based on the number of normal vigorous sprouts (NK) on the day between the first count and the second count in the germination test (Sadjad, 1993). In chili seeds, the simultaneity of growth was observed on the 10th and 11th days, which were then averaged. The simultaneity of growth is calculated using the formula:

$$K_{ST} = \frac{(\Sigma\text{sprout NK days 10} + \Sigma\text{sprouts NK days} - 11)\ /2)}{\Sigma\, Planted\ seeds}\ x\ 100\%$$

2.1.6. Normal/BKKN Dry Weight of Sprouts (g)

The dry weight of normal sprouts was calculated at the end of the germination test observation on the 14th day. All normal sprouts were removed, wrapped in an envelope, and dried in an oven at 80 ºC for 24 hours. The sprouts were placed in a desiccator for 30 minutes and then weighed. This test was carried out at the end of the observation, after the germination observation was completed.

2.1.7. Sprout Growth Rate (g/ g/normal sprout)

The sprout growth rate (SGR) is the ratio between the dry weight of normal sprouts (BKKN) and the number of normal sprouts. The sprout growth rate is calculated using the formula:

$$LPK = \frac{\text{BKKN}}{\Sigma\text{ Normal Sprouts}}$$

2.2. Radicle Emergence Test

The radicle emergence test was conducted by planting 50 seeds on filter paper and placing them on an electric germinator at 20-30°C. The calculation was carried out on seeds with roots with a minimum length of 2 mm (ISTA, 2018), and observations were made every 24 hours, for 168 hours (Kusumawardana et al., 2019). The radical emergence test was calculated using the formula:

$$\%RE = \frac{\Sigma\text{ emerging radicle}}{\Sigma\text{ Sprouted seeds}} x100\%$$

2.3. Electrical Conductivity Test (DHL)

DHL testing was conducted by soaking 100 seeds in 75 ml of aquabidest (ISTA, 2018) at a temperature of 20 ± 2°C for 12 hours, with four repetitions. After the seeds were soaked for 12 hours, the DHL value was measured using a conductivity meter. Measurements were made by measuring the blank solution and the seed soaking water. Measurements were made until a stable number was obtained. The DHL value per gram of seed was calculated using the following formula (ISTA, 2018):

$$\text{DHL}\ (\mu\text{S}\ cm^{-1}\ g^{-1}) = \frac{\text{DHL value of seed soaking water} - \text{DHL Blank Seed weight}}{Seed\ weight}$$

Data analysis was performed using Pearson Correlation Analysis, Analysis of Variance (ANOVA), and Duncan's Multiple Range Test (DMRT) if the treatment significantly affected the response at a 5% significance level. Data analysis was performed using Microsoft Excel 2021 and SAS v9.0 software.

3. Results and Discussion

3.1. Weight of 1000 Seeds

The calculation of 1000 grain weight aims to determine the need for seeds in one hectare for the next planting season. Seeds with a heavier weight have a high endosperm content and are suitable for seed growth (Fajrina & Kuswanto, 2019). One hundred chili seeds were taken from each variety 8 times and then weighed (Table 1). The weighing results were then converted to a weight of 1000 grains. The weight of 1000 grains between recorded varieties varied between 3.65 and 5.33 g.

The highest 1000-grain weight was found in the Feira Variety seeds (5.33 g), while the lowest value was found in the Harita Variety. The greater the 1000-grain weight, the higher the weight of each seed (Kurniawan & Azmi, 2021). The success of farmers' chili production depends on the quality of chili seeds and good cultivation techniques. High-quality seeds are essential in agriculture. Germination, physical purity, and water content indicate seed quality. In addition, it is also seen from the weight of a thousand seeds. In addition, germination and the weight of 1000 seeds can predict the actual seed requirements per land area (Azmi et al., 2023). The role of genetic factors exceeds the environment in influencing the 1000-grain weight variable (Nizar & Mulani, 2015; Jalili & Eyvazi, 2015). Similar research results show that varieties affect the 1000-grain weight in corn, sesame, and cabbage plants. (Devi & Sharma, 2018; Rameeh, 2019; Ramazani, 2016; Jalili & Eyvazi, 2015). Haryati et al. (2024) stated in their writing regarding the effect of differences in harvest time on the quality of chili seeds that the determination of the weight of 1000 chili seeds ranges between 4.8 g and 7.2 g. The results of the study by Sanjuan-Martínez et al. (2020) on the quality of three types of chili seeds (*Capsicum annuum* L.) native to Oaxaca, Mexico, showed that the determination of the weight of 1000 seeds ranged between 4.48 g and 8.21 g.

Table 1. Data on the weight of 1000 grains of 4 varieties of *C. frutescens* chili

Varieties	Weight 100 grains (g)	Weight 1000 grains (g)	Standard Deviation	Variety	Coefficient of Diversity
Beautiful	0.47	4.66 ±0.05 b	0.01	0.0002	0.0302
White chakra	0.41	4.08 ±0.06 c	0.02	0.0003	0.0410
The Way	0.53	5.33 ±0.04 a	0.01	0.0001	0.0194
The Day	0.37	3.65 ±0.04 d	0.01	0.0001	0.0327

Description: Numbers followed by the same letter in the same column indicate no significant difference in the DMRT Test with a significant level of 0.05. The weight data of 1000 seeds were determined in 4 replications.

3.2. Seed Viability and Vigor Testing

Seed viability and vigor are parameters of the physiological quality of seeds. Seed viability is the seed's life force that can be affected by the seed growth process (Tefa, 2017). Pulungan et al. (2025) stated that seed viability is the seed's vitality, metabolically active, and has enzymes that can catalyze metabolic reactions needed for germination and shoot growth. The seed germination power benchmark for growth potential, supported by the germination rate, indicates good seed vigor and viability (Mustakim et al., 2017). Wahyudi (2024) stated that seeds with strong vigor show better storage power. The results of the ANOVA test showed that the variety had a very significant effect on all variables other than Maximum Growth Potential (not substantial) and Germination Power (important) (Table 2). Seed viability testing includes Germination Power (DB) and Maximum Growth Potential (PTM). In contrast, seed vigor testing includes Vigor Index (IV), Growth Rate (KcT), Growth Simultaneity (KsT), Normal Germination Dry Weight (BKKN), and Germination Growth Rate (LPK).

Table 2. ANOVA Recapitulation Results of viability and vigor testing of seeds of 4 varieties of *C. frutescens* chili

No.	Variable	F Value Calculate Variety	Real Level
1	Germination Power	4.22	*
2	Maximum Growth Potential	1.13	tn
3	Vigor Index	31.06	**
4	Growth Rate	30.17	**
5	The Simultaneity of Growth	11.71	**
6	Normally Dry Weight of Sprouts	8.45	**
7	Sprouts Growth Rate	21.96	**
8	Radicle Emergence	9.13	**
9	Electrical Conductivity	70.39	**

Description: * = significantly different at α 0.05, ** = significantly different at α 0.01, tn = not significant

Duncan's Multiple Range Test DMRT with a 5% significance level was conducted on variables with a significant response to the variety. This test aims to determine which variety has the best value. The test results showed that the Harita variety was superior overall in almost all tests (Table 3).

Table 3. Results of the DMRT test of seeds of 4 varieties of *C. frutescens* chili

Varieties	Variable						
	DB	PTM	IV	KcT	KsT	BKKN	LPK
Beautiful	74.50 ±6.99 b	84.50 ±4.57	2.50 ±1.89 b	6.44 ±0.74 c	23.75 ±5.50 c	0.06 ±0.006 b	0.002 ±8.10 $^{-5}$ bc
White chakra	76.50 ±4.99 b	85.00 ±1.73	6.00 ±2.58 b	7.93 ±0.90 bc	55.75 ±11.66 b	0.06 ±0.003 b	0.002 ±6.10 $^{-5}$ b
The Way	85.50 ±2.87 ab	87.00 ±3.51	14.50 ±3.10 b	9.01 ±0.60 b	53.25 ±11.40 b	0.08 ±0.002 a	0.002 ±4.10 $^{-5}$ a
The Day	91.50 ±1.26 a	64.50 ±18.39	51.50 ±7.63 a	12.76 ±0.62 a	87.00 ±2.65 a	0.06 ±0.001 b	0.001 ±3.10 $^{-5}$ c

Description: Numbers followed by the same letter in the same column indicate no significant difference in the DMRT Test with a significant level of 0.05. DB = DB=Germination Power, PTM = Maximum Growth Potential, IV=Vigor Index, KcT=Growth Speed, KsT=Growth Simultaneity, BKKN=Normal germination dry weight, LPK=Sprout Growth Rate.

Seeds have different physical characteristics. Seeds with a testa that is permeable to water increase the rate of imbibition in seeds, accelerating the seed germination process. Seeds that have a hard testa take longer to germinate than other seeds. The research results by Haryati et al. (2024) stated that chili seeds harvested in the third and fourth weeks had the highest maximum growth potential values, 94.5% and 93.0%, respectively.

3.3. Seed Quality Testing In Laboratory-RE

Vigor testing is carried out to evaluate seed quality effectively and efficiently. The Radicle Emergence (RE) test is a rapid seed vigor testing method based on the International Rules for Seed Testing. The RE test results on chili seeds showed that Harita had the highest RE value (92.00). (Table 4).

Table 4. Results of the Radicle Emergence Test of seeds from 4 varieties of *C. frutescens* chili

Varieties	*Radicle Emergence*				
	U1	U2	U3	U4	Average
Beautiful	16.0	34.0	30.0	50.0	32.50 ±6.99 c
White chakra	38.0	36.0	82.0	86.0	60.50 ±13.60 b
The Way	56.0	54.0	90.0	46.0	61.50 ±9.74 b
The Day	92.0	88.0	98.0	90.0	92.00 ±2.16 a

Description: Numbers followed by the same letter in the same column indicate no significant difference in the DMRT test with a significant level of 0.05.

Astuti et al. (2020) showed that the RE test was closely correlated and could predict the percentage of germination power, vigor index, growth rate, average germination time, growth power, and seedling emergence time. This shows that the RE variable can be used as a rapid seed vigor test. Wirianto et al. (2024) stated that, from the results of their study, the radicle length category suitable for developing a chili seed vigor test method is ≥ 1 mm and ≥ 2 mm at intervals of 84 and 93 hours, respectively. The radicle length category ≥ 50% PJB and ≥ PJB do not strongly correlate with the percentage of germination power in the nursery.

3.4. Electrical Conductivity of Seeds

Electrical conductivity (EVC) of seeds is a variable that can detect seed vigor by knowing seeds' decline through seed coat leakage (Figure 2). The EVC test is one of the tests to estimate the speed of germination and seed vigor. This test is carried out to determine the electrical conductivity value of seed soaking water, indicating the level of electrolyte leakage from the cell membrane in the seeds.

The DHL test results showed that the Harita variety had the lowest potential leakage rate, 173.80 (Table 5). Larassati et al. (2014) stated that seeds with low DHL values illustrate that the seeds have high vigor and vice versa.

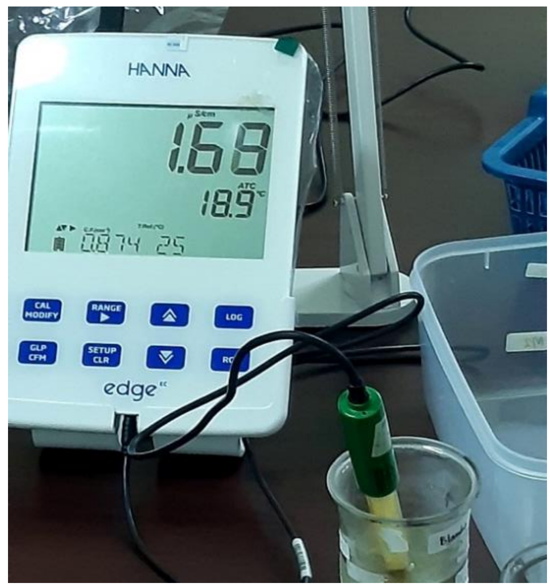

Figure 2. Electrical conductivity test of *C. frutescens* chili seeds.

Table 5. Results of Electrical Conductivity (EDC) Test of seeds from 4 varieties of *C. frutescens* chili

Varieties	DHL value (μS cm-1 g-1)				
	U1	U2	U3	U4	Average
Beautiful	189.18	205.52	205.82	201.74	200.57b
White chakra	264.74	243.28	250.89	274.49	258.35a
The Way	201.96	207.09	215.37	212.91	209.33b
Harita	173.09	169.29	172.72	180.11	173.80c

Description: Numbers followed by the same letter in the same column indicate no significant difference in the DMRT test with a significant level of 0.05.

Noviana et al. (2016) stated that cell membrane damage is caused by changes in protein and lipid structures through hydrolysis and oxidation processes, causing membrane leakage with some electrolytes coming out, which are then measured through electrical conductivity values that can be detected by a conductivity meter.

3.5. Correlation Between Observation Variables

Pearson Correlation Analysis was conducted on all observation variables. The results of the analysis showed a very close relationship ($r>0.8$), significantly positive between the RE variable and the DB, KcT, and KsT variables (0.85, 0.94, and 0.99, respectively). Similar relationships were also found between the KsT variable and the DB, KcT variables (0.80, 0.92, respectively), between the KcT variables and DB, IV (0.86 and 0.88, respectively) (Table 6).

The results of the study by Jawak et al. (2022) related to the estimation of seed deterioration through physiological and biochemical tests showed a significant positive relationship between the Germination Power variable and IV of 0.98. Kusumawardana et al. (2019) stated the conclusions of their research related to testing the quality of chili seeds using the radicle emergence test method, namely that there is a very close correlation between the radicle emergence test and germination power (r = 0.907), vigor index (r = 0.864) and growth rate (r = 0.727).

Table 6. Results of the correlation analysis between the observation variables of the viability test and seed vigor.

Variable	DB	PTM	IV	KcT	KsT	BKKN	LPK	RE
PTM	-0.13							
IV	0.56*	-0.62*						
KcT	0.86*	-0.45	0.88*					
KsT	0.80*	-0.34	0.70*	0.92*				
BKKN	0.61*	0.32	-0.04	0.28	0.29			
LPK	-0.27	0.38	-0.43	-0.41	-0.33	0.54*		
RE	0.85*	-0.29	0.69*	0.94*	0.99*	0.34	-0.35	
DHL	-0.33	0.3	-0.61*	-0.45	-0.18	0.02	0.34	-0.2

Description: * = significant correlation at the α = 5%. DB = Germination Power, PTM = Maximum Growth Potential, IV = Vigor Index, KcT = Growth Speed, KsT = Growth Simultaneity, BKKN = Normal Germination Dry Weight, LPK = Germination Growth Rate, RE = Radicle Emergence, DHL = Electrical Conductivity.

4. Conclusion

The Feira variety of *C. frutescens* seeds had the highest 1000-grain weight at 5.33 g, whereas the lowest value was recorded in the Cakra Putih variety at 4.08 g. According to the DHL test results, the Harita variety exhibited the lowest potential leakage rate at 173.80. The Harita variety showed the highest vigor value in the Vigor Index, Growth Rate, Growth Simultaneity, and Sprout Growth Rate. The Pearson Correlation analysis results indicated a strong, positive correlation (r>0.8) between RE variables and DB, KcT, and KsT variables.

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