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Seed Diversity Five Species of Chili (*Capsicum* spp.) Based on Morphological Characters and Seed Viability

Keanekaragaman Benih Lima Spesies Cabai (Capsicum spp.) Melalui Karakter Morfologi dan Viabilitas Benih

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ABSTRAK

Benih cabai memiliki banyak keragaman dan merupakan salah satu kunci keberhasilan dalam budidaya. Identifikasi keragaman benih cabai, pengujian viabilitas benih diperlukan sebagai langkah dalam perakitan varietas dan penyediaan benih bermutu. Penelitian bertujuan memperoleh informasi bentuk biji, tonjolan paruh, bobot 1000 biji dan viabilitas benih. Penelitian dilakukan terhadap 63 genotipe cabai dari 5 spesies (Capsicum annuum, C. frutescens, C. chinense, C. baccatum, dan C. pubescens) bulan Februari-Juli 2023 di Laboratorium Ilmu dan Teknologi Benih IPB, menggunakan rancangan kelompok lengkap teracak 3 ulangan. Pengamatan meliputi sifat morfologi dan fisiologi benih cabai. Biji cabai secara umum berwarna putih kekuningan dengan tonjolan paruh, kecuali C. pubescens (warna biji hitam, tidak ada tonjolan paruh), bentuk biji seperti ginjal (C. annuum), lonjong (C. baccatum), tetes air mata (C. frutescens), berbentuk D (C. pubescens), melingkar dengan mulut ikan (C. chinense). Spesies cabai yang diamati memiliki berbagai ukuran bobot 1,000 biji (ringan (<3.90 g), sedang (3.90-5.68 g), dan berat (>5.68 g). Keragaman genetik perkecambahan, potensi tumbuh maksimum, indeks vigor, kecepatan tumbuh, keserempakan tumbuh berkategori luas, sedangkan bobot kering kecambah normal, laju pertumbuhan, bobot 1000 biji berkategori sempit. Ukuran biji tidak mempengaruhi persentase perkecambahan benih. Spesies C. pubescens memiliki karakteristik benih yang berbeda dengan spesies Capsicum lainnya, sedangkan spesies C. annuum memiliki kemiripan dengan C. frutescens, C. chinense, dan C. baccatum.

Kata kunci: benih, cabai, keanekaragaman, morfologi, spesies

ABSTRACT

Chili seeds have a lot of diversity and are a cultivation success. Identification of chili seed diversity and seed viability testing is needed as one step in assembling varieties and providing quality seeds. The objective of this study was to obtain information on seed shape, beak protrusion, 1000-seed weight, and seed viability. The study was conducted on 63 chili genotypes from 5 species (*Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*) in February-July 2023 at the IPB Seed Science and Technology Laboratory, using a randomized complete block design with 3 replications. Observations include the morphological and physiological properties of chili seeds. Chili seeds are generally yellowish white with a beak protrusion, except *C. pubescens* (black color, no beak protrusion), kidney-shaped seeds (*C. annuum*), oval (*C. baccatum*), teardrops (*C. frutescens*), D-shaped (*C. pubescens*), circular with a fish mouth (*C. chinense*). The chili species observed had various sizes of 1000-seed weight (light (<3.90 g), medium (3.90-5.68 g), and heavy (>5.68 g). Genetic diversity of germination, maximum growth potential, vigor index, growth rate, and growth simultaneity were categorized broadly, while normal dry weight of sprouts, growth rate, and 1,000 seed weight were categorized narrowly. Seed size did not affect the percentage of seed germination. The *C.pubescens* species had different seed characteristics from other Capsicum species, while the *C.annuum* species had similarities with *C.frutescens*, *C.chinense*, and *C.baccatum*.

Keywords: chili, diversity, morphology, seeds, species

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INTRODUCTION

The world community considers chili (*Capsicum* spp.) as one of the important horticultural commodities. The use of chili can be found in every aspect of human life. Chili has various species, but until now only five species are commonly used and consumed, namely: *Capsicum annuum*, *C. frutescens*, *C. baccatum*, *C. chinense*, and *C. pubescens*. Initially, chili was used as a cooking spice, then developed as a biopharmaceutical ingredient that utilizes capsaicin content (Sahid et al., 2020). The capsaicin content in the five chili species is based on the Scoville spiciness unit (SHU) from 0-1.6 million SHU, sorted by spiciness level (*C. chinense* > *C. pubescens* > *C. baccatum* > *C. frutescens* > *C. annuum*).

The World Vegetable Center (WVC)/The Asian Vegetable Development and Research Center (AVRDC) stored 286 accessions of chili pepper in 1986. Over the past 30 years, this number has increased, and WVC's collection now contains 8,264 accessions from at least 100 countries worldwide (Jarret et al., 2019). In addition, The Vegetable Research and Development Center (TVRC), Kasetsart University, Thailand, has also collected more than 2827 chili accessions from all over Thailand (Mongkolporn et al., 2015). Most of these accessions have been characterized conventionally and also using molecular markers for various characters (Munoz-Concha et al., 2020), such as agronomic characters, fruit quality, and resistance to virus attack (Nankar et al., 2020; Sayekti et al., 2021). However, there has yet to be much research on seed characterization, as reported by Chiou and Hastorf (2014) and Kurniawan and Azmi (2021).

Seed characterization is needed to determine the initial identification of the chili species to be cultivated. Chili production success is significantly influenced by seed quality and appropriate cultivation techniques. Quality seeds are an important concern in agriculture because seeds are the main input and the first chain in plant cultivation (Widisatriani et al., 2015). Chili production is also related to the availability of quality seeds. Seed, as a commercial product, must have clear quality standards. The continuous availability of quality seeds is important because it determines the success of production in quality and quantity. The use of quality seeds prevents farmers from losses. Some of the problems in seed germination are inhibitory substances, including the capsaicin content (Barchenger and Bosland, 2016) and the pollen originating from the parents used (Naves et al., 2022).

Therefore, quality seeds are very important in agricultural cultivation activities. Seed quality is commonly evaluated based on germination percentage and 1,000-seed weight. These two characteristics can help predict the seed requirements for a given planting area. Seed quality can be reflected in its ability to grow in the field. One of the characteristics of seed quality can be estimated based on germination tests in the laboratory. Germination tests in the laboratory are expected to provide information about potential germination in the field (Soleymani, 2019), while the test results are expected to describe the conditions for plant growth in a wider planting area (Marcos-Filho, 2015). Tests in the laboratory must have a positive correlation with plant growth in the field (Nikolić et al., 2021), so that the results of laboratory tests can be used as a reference for testing early growth.

The Plant Breeding Laboratory of Department of Agronomy and Horticulture, Bogor Agricultural University has a collection of five chili species and more than 63 chili genotypes that have the potential to be further developed. However, information on the morphological and physiological of the genotypes from these collections has yet to be studied. Lestari et al. (2024), reported that drought stress can cause a decrease in chili productivity and cause economic losses. Undang et al. (2024), reported that information on the morphology and physiology of chili seeds is needed to identify the germination properties of chili, one of which uses a genetic parameter approach with the Hayman diallel method. This study was to determine the genetic diversity based on the morphological and physiological characteristics of the seeds of five different chili species, namely *Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens*. The results of this study are expected to enrich the information on the differences between chili species, with information on the morphological and physiological characters of the seeds.

MATERIALS AND METHODS

Study Laboratory and Genetic Material

The research was conducted at the Seed Science and Technology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University (IPB), from February to July 2023. This study tested chili seeds from five different species: *Capsicum annuum* (41 genotypes), *C. frutescens* (10 genotypes), *C. chinense* (9 genotypes), *C. baccatum* (2 genotypes) and *C. pubescens* (1 genotype) which are collections of the Center for Tropical Horticultural Studies IPB and the Genetics and Plant Breeding Laboratory, Department of Agronomy and Horticulture, IPB University (Table 1).

Procedure

The research began by planting the test genotypes in the CV Benih Darmaga greenhouse, Cibeureum, Bogor, as a source of research seeds. The seeds tested came from the same growing season. Seeds of each genotype were used as test material for seed morphological characterization and physiological quality testing. All observations made were destructive.

Morphological characterization was carried out by visually observing seed color, seed shape, protruding seed beak, and 1000-seed weight. The criteria for the shape of the seeds and the protruding beak of the chili seeds refer to Chiou and Hastorf (2014). Determination of the 1000-seed weight data was calculated from the weight of 100 seeds with

Table 1. The genetic material of 63 chili pepper genotypes used in the study

No	Genotype	Seed color	Source	No	Genotype	Seed color	Source
C. annuum				34	F13.120005	yellowish-white	IPB
1	Adelina	yellowish-white	IPB	35	F14.160291	yellowish-white	IPB
2	Anies	yellowish-white	IPB	36	F7.110005	yellowish-white	IPB
3	Arisa	yellowish-white	IPB	37	F8.074035	yellowish-white	IPB
4	Ayesha	yellowish-white	IPB	38	F8.074077	yellowish-white	IPB
5	Bara	yellowish-white	Ewindo	39	F8.074136	yellowish-white	IPB
6	Chenzo	yellowish-white	Meksiko	40	F8.136074	yellowish-white	IPB
7	Fish Pepper	yellowish-white	Amerika	41	F9.074	yellowish-white	IPB
8	Genie	yellowish-white	Benih Citra Asia	*C. frutescens*			
9	Hot Banana	yellowish-white	Amerika	42	Bonita	yellowish-white	IPB
10	Imperial	yellowish-white	BISI	43	Cakra Putih	yellowish-white	BISI
11	Jelita	yellowish-white	IPB	44	Cibeureum	yellowish-white	IPB
12	Lembayung	yellowish-white	IPB	45	Cimanggu	yellowish-white	IPB
13	Namira	yellowish-white	IPB	46	Feira	yellowish-white	IPB
14	Nazla	yellowish-white	IPB	47	Harita	yellowish-white	IPB
15	Neno	yellowish-white	IPB	48	Rawit 4H-1	yellowish-white	IPB
16	Pesona	yellowish-white	IPB	49	F10.285290	yellowish-white	IPB
17	Pesona 2	yellowish-white	IPB	50	F11.285290	yellowish-white	IPB
18	Peter Pepper	yellowish-white	Amerika	51	F11.321290	yellowish-white	IPB
19	Purple Candle	yellowish-white	Brazil	*C. chinense*			
20	Seloka	yellowish-white	IPB	52	Habanero Fransisca	yellowish-white	Amerika
21	Seloka 4	yellowish-white	IPB	53	Katokon	yellowish-white	Local Toraja
22	Seroja	yellowish-white	IPB	54	Orange Chupetinho	yellowish-white	Brazil
23	Shiara	yellowish-white	IPB	55	Orange Habanero	yellowish-white	Amerika
24	Solar Fiare	yellowish-white	IPB	56	Peach Chupethinho	yellowish-white	Brazil
25	SSP LKP	yellowish-white	IPB	57	Red Bhut Jolokia	yellowish-white	India
26	SSP-1-2	yellowish-white	IPB	58	Red Chupetinho	yellowish-white	Brazil
27	Syakira	yellowish-white	IPB	59	Red Habanero	yellowish-white	Amerika
28	Triwarsana	yellowish-white	IPB	60	Thai Pumkin	yellowish-white	Thailand
29	Ungara	yellowish-white	IPB	*C. baccatum*			
30	Viola	yellowish-white	IPB	61	Bishop Crown	yellowish-white	Brazil
31	Violeta	yellowish-white	IPB	62	Lemon Drop	yellowish-white	Peru
32	F12.145174	yellowish-white	IPB	*C. pubescens*			
33	F12.145291	yellowish-white	IPB	63	Gendot	black	Local Java

eight replications according to the standard procedures of the International Seed Testing Association Rules (ISTA, 2021; Kurniawan and Azmi, 2021). Morphological images of chili seeds were taken with a "dinolite" microscope connected to a monitor with 40x magnification.

Testing the physiological quality of chili seeds consists of testing the viability and vigor of chili seeds. The two tests were carried out according to ISTA rules (2021), namely testing with the top-of-paper method. Seed viability and vigor tests were performed on 50 seeds per genotype in a randomized complete block design with three replications. Observations included seed germination characteristics, maximum growth potential, vigor index, growth speed, growth uniformity, normal seedling dry weight, and growth rate of normal seedlings.

The germination of chili seeds was carried out in a closed plastic box using media in the form of three sheets of CD paper moistened with distilled water. The germination box was placed in an electric germinator with a temperature of 28 °C with a measuring device and thermometer and using a moist seedling medium.

Data Analysis

Qualitative data were analyzed descriptively according to the criteria Chiou and Hastorf (2014). Grouping the 1000-seed weight into three weight categories: light, medium, and heavy, following Walpole, (1982) with the formula:

with ΔP = change in yield of a variable, n = number of groups of seed weight, a = minimum value of seed weight, b = maximum value of seed weight, x = observed value.

The seed weight classes were defined as follows:

light = $x < \Delta P + a$

medium = $\Delta P + a < x < (\Delta P + a) + \Delta P$

heavy = $x > (\Delta P + a) + \Delta P$

Analysis of variance was performed following the procedure of Steel and Torrie (1981). Estimation of genetic parameters and heritability was performed following the methods of Lush (1949) and Johnson et al. (1955) (Table 2):

$\sigma^2G = (M2-M3) / r$

$\sigma^2e = M3$

$\sigma^2P = \sigma^2G + \sigma^2e$

$h^2bs = (\sigma^2G / \sigma^2P) \times 100\%$

The percentage criteria for heritability according to Whirter (1979) are: low ($0 < x < 20\%$), medium ($20 \leq x < 50\%$) and high ($x \geq 50$). The criteria for genetic variation > 2x standard deviation of genetic variation is wide, while the criteria for genetic variation < 2x standard deviation of genetic variation is narrow.

Genotype grouping based on morphological and physiological seed traits was performed using clustering analysis with the PBSTAT-CL 2.1.2 application. The specific grouping based on the percentage of germination (germination adjusted) and the 1000-seed weight (1000-seed weight adjusted) was done based on value of standard deviation of each data unit to mean value, as an adjusted value of each character, through the equation =((x_i-x⁻))/SD, with x_i = per data unit, (x)‾ = general mean, SD = standard deviation, analysis by Minitab v.19.

RESULTS AND DISCUSSION

Seed Shape

The results of seed morphological observations provide information that chili seeds have a round shape, but each chili species has different round specifications. The diversity of seed shapes of the five chili species is presented in Figure 1. In general, *Capsicum annuum* genotypes had reniform-shaped seeds identified from 41 genotypes. *C. baccatum* seeds are oval (2 genotypes), *C. frutescens* seeds are teardrop-shaped (10 genotypes), *C. pubescens* seeds are D-shaped (1 genotype), and *C. chinense* seeds are circular with a fish-mouth structure (9 genotypes).

Beak Prominence

The beak prominence reflects the orientation of the hilum protruding from the body of the seed. The beak

Table 2. F-Ratios were used to test the effects of randomized complete block design experiments

Sources of variation	Mean square	Expected mean squares (fixed model)
Replication (Rep)	M1	-
Genotype (Geno)	M2	$\sigma^2e + r\sigma^2_G$
Error	M3	σ^2e

Note: σ^2e: environmental variance; σ^2_G: genotypic variance

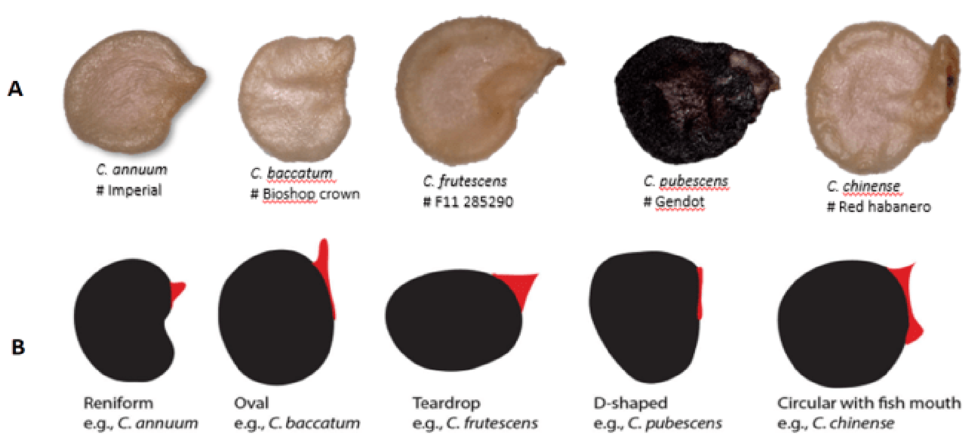

Figure 1. The diversity of seed shapes of Capsicum spp.: A. seed shapes of each species with examples of test genotypes (using a 'dinolite' microscope at 40x magnification), B. seed shapes of Capsicum according to Chiou and Hastorf

prominence is distinguished from no beak (scale 1, none) to a very prominent vertical beak (scale 5, large). The five Capsicum species tested showed a variety of beak prominence at scales 1, 2, and 4. The seeds of *C. pubescens* were the most different from the other four tested species because they did not have a beak prominence (scale 1). The three species tested, namely *C. baccatum*, *C. frutescens*, and *C. chinense*, have a medium beak prominence type (scale 4). Based on Chiou and Hastrof (2014) in Figure 2B, scale 4 (medium) shows longer beak prominence than scale 3 (small) but shorter than scale 5 (large). The only species with scale 2 (nub type) beak prominence was *C. annuum*. The nub type has a slight beak prominence. The visual scale of the beak prominence of the five species tested is shown in Figure 2A, which refers to the description by Chiou and Hastorf, (2014) in Figure 2B.

The 1000-seed Weight

Sixty-three genotypes tested of five chili species have 1000-seed weight ranges from 2.13-7.45 g (Table 3). The 'Jelita' genotype had the smallest weight (2.13 g), while the 'Gendot' genotype (*C. pubescens*) had the highest weight (7.45 g). That result is similar to research by Nagaraju et al. (2018) that the variation in trait 1000-seed weight chili ranges from 3.38 - 10.07 g, while Kurniawan and Azmi (2021) reported ranges from 4.13 - 7.64 g.

The test genotypes were grouped as heavy seeds (>5.68 g) 12 genotypes, medium (3.90-5.68 g) 42 genotypes, and light (<3.9 g) 9 genotypes. Seeds of *C. annuum* species vary in size: light, medium, and heavy, likewise with the species *C. frutescens* and *C. chinense*. Species *C. baccatum* has a medium weight, while *C. pubescens* is heavy. Most seeds of the species *C. annuum*, *C. frutescens*, *C. chinense* and *C. baccatum* are medium weight. Differences in the 1000-seed weight among chili varieties within the same species have also been reported by Nalwa and Kumar (2019) and Kurniawan and Azmi (2021).

Genetic Diversity and Heritability of Five *Capsicum* Species

The analysis results of genetic diversity and broad-sense heritability are presented in Table 4. The heritability values of all seed morphological and physiological characters were high (74.54% to 97.73%), except for normal seedling dry weight (14.80%, low) and seedling growth rate (0.00%, low). The broad-sense heritability value indicates a major role of genetic factors controlling the character. The genetic diversity of the 1000-seed weight character was narrow, while the physiological character was wide (2,538 – 34,537), except for normal seedling dry weight (0.021, narrow) and seedling growth rate (0.00, narrow).

Classification of 63 Chili Genotypes Based on Seed Morphological and Physiological Characters

The grouping of 63 chili genotypes from five Capsicum species was performed based on morphological traits (1000-seed weight) and all observed seed physiological characteristics (germination, maximum growth potential, vigor index, growth rate, simultaneous growth, normal seedling dry weight, and seedling growth rate. The grouping of all tested genotypes is shown in Figure 3. Based on a similarity threshold of 0.5, the tested chili genotypes were grouped into four clusters.

The chili genotypes of *C. annuum* and *C. frutescens* were grouped in cluster I, II, and IV. Most of the chili genotypes of *C. annuum*, including 35 genotypes, were grouped in cluster II. The species *C. chinensis* and *C. baccatum* only clustered in cluster IV, which also grouped with several genotypes from *C. annuum* and *C. frutescens*, and *C. pubescens* was grouped in cluster 3. Genotypes that were grouped in the same cluster indicated a closer similarity.

In more detail, cluster I consisted of 8 genotypes, namely three *C. annuum* genotypes ('SSP-1-2', 'Ungara', 'F8-074136') and five genotypes of *C. frutescens* ('Bonita',

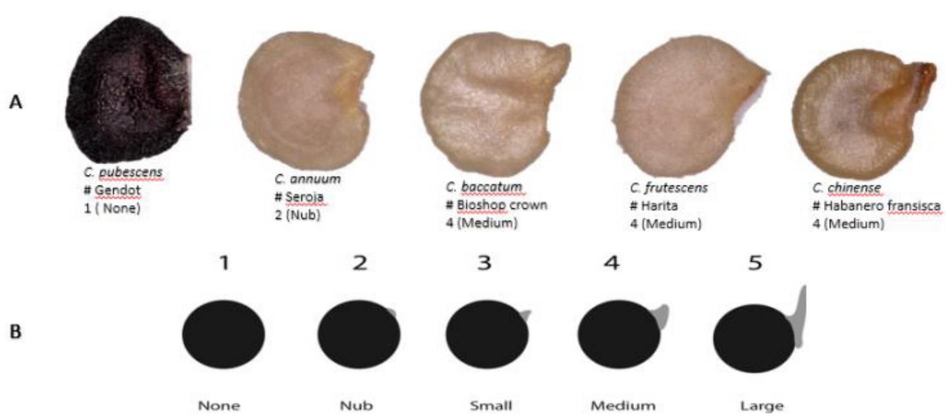

Figure 2. Ranking scale of beaks prominence of five chili species: A. the type of the beak prominence and examples of the test genotypes of each species (using a ‘dinolite’ microscope at 40x magnification), B. the shape of the Capsicum beak prominence by according Chiou and Hastrof (2014).

'Feira', 'Rawit4h-1', 'F10-285290' and 'F11-285290'). Cluster II consisted of 37 genotypes, with 35 genotypes of *C. annuum* (Anies', 'Arisa', 'Ayesha', 'Bara', 'Chenzo', 'Fish pepper', 'Genie', 'Hot banana', 'Imperial', 'Jelita', 'Namira', Nazla', 'Neno', 'Pesona', 'Pesona2', 'Pesona2', 'Peter pepper', 'Purple candle', 'Seloka', 'Seroja 4', 'Seroja', 'Shiara', 'Solar fiare', 'SSP Lewikopo', 'Syakira', 'Triwarsana', 'Violeta', 'F12.145174', 'F12.145291', 'F13.120005', 'F14.160291', 'F7.110005 ', 'F8.074035', 'F8.074077', 'F8.136074', and 'F9.074') and two genotypes of *C. frutescens* ('Cakra putih' and 'Harita'). Cluster III is only one genotype of *C. pubescens* ('Gendot'). Cluster IV consisted of 17 genotypes, with three *C. annuum* genotypes ('Adelina', 'Lambayung', and 'Viola'), three *C. frutescens* genotypes ('Cibeureum', 'Cimanggu', and 'F11.321290'), nine *C. chinensis* genotypes ('Habanero francisca', 'Katokon', 'Orange chupotinho', 'Orange habanero',

Table 3. Mean value and category of 1000-seed weight all genotypes tested from five *Capsicum* species

Genotype	1000-seed weight (g)	Category	Genotype	1000-seed weight (g)	Category
C. annuum			Solar fiare	4.89	medium
Adelina	4.80	medium	Ssp leuwikopo	3.96	medium
Anies	5.41	medium	SSP-1-2	5.08	medium
Arisa selec	6.35	heavy	Syakira	4.13	medium
Ayesha	3.69	light	Triwarsana	4.60	medium
Bara	3.63	light	Ungara	5.38	medium
Chenzo	5.69	heavy	Viola	4.55	medium
F12 145174	4.16	medium	Violeta	4.11	medium
F12 145291	4.20	medium	***C. frutescens***		
F13 120005	4.80	medium	Bonita	4.66	medium
F14 160291	3.54	light	Cakra putih	4.08	medium
F7 1100053	5.41	medium	Cibeureum	6.28	heavy
F8 074035	6.98	heavy	Cimanggu	5.20	medium
F8 074077	5.33	medium	F10 285290	5.39	medium
F8 074136	6.65	heavy	F11 285290	5.20	medium
F8 136074	6.84	heavy	F11 321290	5.88	heavy
F9 074	6.06	heavy	Feira	5.33	medium
Fish pepper	4.91	medium	Harita	3.65	light
Genie	3.40	light	***C. chinense***		
Hot banana	5.54	medium	Habanero fransisca	4.43	medium
Imperial	6.58	heavy	Katokon	4.33	medium
Jelita	2.13	light	Orange chupetinho	3.19	light
Lembayung	3.99	medium	Orange habanero	4.84	medium
Namira	4.19	medium	Peach chupethinho	3.29	light
Nazla	5.05	medium	Red bhut jolokia	5.68	medium
Neno	5.44	medium	Red chupetinho	3.09	light
Pesona	5.10	medium	Red habanero	4.98	medium
Pesona 2	6.54	heavy	Thai pumkin	4.53	medium
Peter pepper	6.75	heavy	***C. baccatum***		
Purple candle	5.39	medium	Bioshop crown	4.53	medium
Seloka	4.48	medium	Lemon drop	4.08	medium
Seloka 4-10	4.31	medium	***C. pubescens***		
Seroja	4.16	medium	Gendot	7.45	heavy
Shiara	4.23	medium			

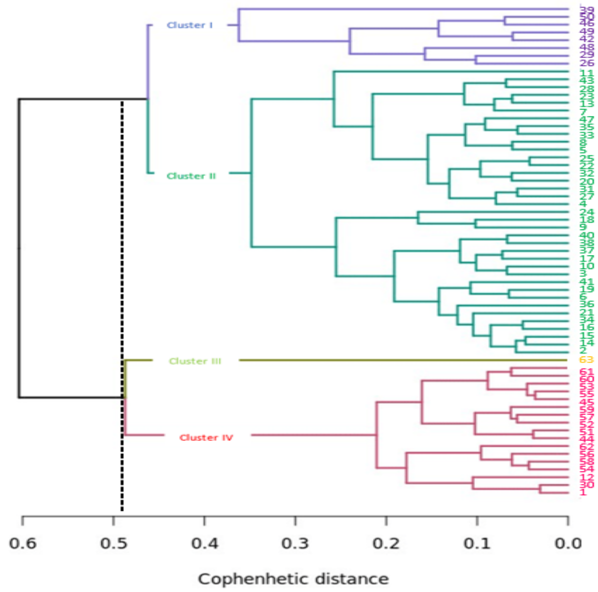

Figure 3. Relationship dendrogram between chili genotypes using seed morphology and physiology characters. (Note. Description of genotypes (numbers) according to Table 1)

'Peach chupotinho', 'Red chupotinho', 'Red habanero', 'Thai pumkin', and 'Red bhut jolokia'), and two genotypes of *C. baccatum* ('Bioshop crown', and 'Lemon drop').

Grouping based on 1000-seed weight and germination characters, 63 genotypes chili tested were grouped into four different quadrants (Figure 4). The 63 chili genotypes were classified into four quadrants based on 1000-seed weight and germination rate: quadrant I (light seed weight, low germination rate), quadrant II (light seed weight, high germination rate), quadrant III (heavy seed weight, high germination rate), and quadrant IV (heavy seed weight, low germination rate).

Discussion

Genetic factors (G), environmental (E), and interaction GxE affect character phenotype and influence phenotypic expression (Table 4). These character differences help define the identity of a genotype. Character differences will be more and more in different species. Previous studies on chili plants have focused primarily on morphological and physiological characterization, with limited research on seed traits. Several studies on genetic diversity have identified that it is limited to parts of stems, leaves, flowers, fruit, resistance to viruses, capsaicin content, and other metabolite contents, as reviewed by Sahid et al. (2020); Sanjuan-Martínez et al. (2020); Sayekti et al. (2021); Li et al. (2022); Cavasin et al. (2023). Only a few studies reported the identification of chili seeds, as was done by Chiou and Hastorf, (2014) and Azmi et al. (2023).

Seed is a planting material that determines the quality of life of a plant. Sadjad (1993) defines seeds to develop farming and have an agronomic function. Therefore, seeds must be of high quality or superior because seeds must be able to produce new plants that can grow maximum, especially when supported by increasingly advanced technological facilities. Seed quality determines the success of a research program and is even seriously considered in the commercial business of seeds by seed companies. As a first step in seed quality determines, it is necessary to know the character differences within and between chili species.

Chili seeds generally have a general yellowish-white color. In this study, one genotype displayed a distinctly different seed color black. The Gendot genotype (*C. pubescens*) was the only one with black seeds (testa color). Out of a total of 41 genotypes of *C. annuum*, no other distinct color. Thus, seed color has distinguished C. pubescens from other Capsicum species, especially *C. annuum* and *C. frutescens*. This result is in line with the reports by Djarwaningsih, (2005); Chiou and Hastorf, (2014); Undang et al., (2015) that chili seeds are generally yellowish-white in color and only one is black, namely *C. pubescens*.

In general, chili seeds have a round shape, but the five chili species have different round specifications (Figure 1). The *Capsicum annuum* species is 'reniform', *C. baccatum* is 'oval', *C. frutescens* is 'teardrop', *C. pubescens* is 'D-shaped' and *C. chinense* is 'circular with fish mouth'.

The beak shape of five chili species can be seen in Figure 2. The species *C. pubescens* does not have a scale 1 beak prominence, *C. annuum* has a slight scale 2 prominence, while *C. baccatum*, *C. frutescens* and *C. chinense* have a scale of 4 beak prominence. The type of beak prominence can be a characteristic of the species *C. pubescens* and *C. annuum* which can be a differentiator from other species. However, this character has not been able to be a characteristic among the species *C. baccatum*, *C. frutescens* and *C. chinense* which have similar beak prominence shapes. These results are in line with the research of Chiou and Hastrof (2014). Environmental factors have very little influence on qualitative characters so their phenotypes (performance) tend to be the same in various conditions. Qualitative characteristics of chili seeds such as seed color, and type of beak prominence are suitable to be used as characteristics of a chili species and are easy to identify. Characterization of seeds, especially chili, is still rarely reported, including by Chiou and Hastorf (2014) and Azmi et al. (2023). Research on the characterization of chili species mostly reports on the characterization of plant or fruit quality such as stem, leaf, flower, fruit, virus resistance (Sayekti et al., 2021), capsaicin content (Sahid et al., 2020), and chili metabolite content (Cavasin et al., 2023).

In general, *C. annuum* is circular in seed shape (Figure 1). Species of *C. chinense* have a perfectly round shape with a beak prominence marked by a more open slit like a fish's mouth, as same as a 'circular fish mouth' shape. The beak prominence in this species is quite long (medium). It seems

Table 4. Genetic diversity and heritability of seed morphology and physiology characters of five Capsicum species

Character	σ^2G	$2\sigma(\sigma^2G)$	*Criteria of σ^2G	σ^2P	h^2bs (%)	**Criteria of h^2bs
Morphology						
1000-seed weight (g)	0.01	0.106	narrow	0.0114	97.73	high
Physiology						
Germination (%)	283.72	16.844	wide	380.64	74.54	high
Maximum growth potential (%)	1192.79	34.537	wide	1581.00	75.45	high
Vigor index (%)	348.39	18.665	wide	431.79	80.68	high
Growth rate (%)	6.44	2.538	wide	7.78	82.77	high
Simultaneous growth (%)	330.14	18.170	wide	427.53	77.22	high
Normal seedling dry weight (g)	0.00043	0.021	narrow	0.0029	14.80	low
Seedling growth rate (g/normal seedling)	0.0000	0.000	narrow	0.0000	0.00	low

Note: σ^2P: phenotypic variance; σ^2G: genotype variance; 2 $\sigma(\sigma^2G)$: 2 x stdev of genotypic variance; h^2bs: broad-sense heritability,
* if $\sigma^2G > 2\sigma(\sigma^2G)$ is wide, $\sigma^2G < 2\sigma(\sigma^2G)$ is narrow; **low ($0 < x < 20\%$), medium ($20 \leq x < 50\%$), and high ($x \geq 50$).

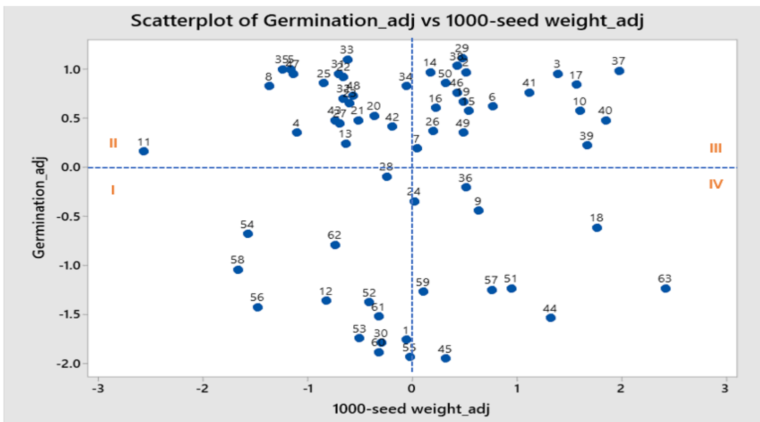

Figure 4. Distribution of 63 chili genotypes based on germination rate and 1000-seed weight. (Note. Description of genotypes (numbers) according to Table 1)

understandable that the circular shape of the chili seeds then undergoes modifications in the narrowing of the horizontal and vertical directions. Then this shape modification characterizes a chili species (Chiou and Hastorf, 2014).

Germination and 1000-seed weight are influenced by genetic diversity. Genetic diversity is very important in plant breeding. The genotypes tested were evenly distributed in all four quadrants. This situation showed different morphological and physiological characteristics of each chili genotype tested. On average, genotypes with heavy seeds have a high percentage of germination, but not a few genotypes with heavy seeds have a low germination rate. Groups of heavy seed sizes (1000-seed weight) with similar harvesting age, even the same species, do not guarantee a high germination percentage. Chili genotypes in quadrants II and III are recommended as potential parents in the hybridization. Combinations of crosses between different clusters can be suggested for breeding programs. Furthermore, it is necessary to test hybrid seeds (F1) or segregated populations (Azmi et al., 2023; Undang et al., 2024).

The role major of genetic factors over a character provides initial information for selection. The proportion of genetic factors was estimated from heritability. Selection is more effective when genetic diversity is high (Usman et al., 2017). The characters for germination rate, maximum growth potential, vigor index, growth speed, and 1000-seed weight have high broad-sense heritability, which indicates that diversity due to genetic differences, as shown by review Usman et al. (2014) and Sayekti et al. (2021). Genetic factors influence the weight of 1000 grains more than the environment (Jalili and Eyvazi, 2015; Nizar and Mulani, 2015). Environmental factors that also affect 1000-seed weight are the management of plant nutrition (Angelopoulou et al., 2019; Kumar et al., 2019; Verma and Mehta, 2019; Zangani et al., 2021; Kristó et al., 2023), micro fertilizers (Nature and Islam, 2016; Soheili-Movahhed et al., 2019), water (Solichatun et al., 2022), temperature (Thuy and Kenji, 2015), priming and pelleting (Verma and Mehta, 2019; Rhaman et al., 2020), plant spacing (Can et al., 2020; Janni et al., 2023), planting time (Devi and

Sharma, 2018), pollination method (Aminatun et al., 2019), planting method (Rameeh, 2019), and plant population (Wakweya et al., 2016; Ninou et al., 2019). Therefore, there is high genetic diversity in germination characteristics, maximum growth potential, vigor index, and growth speed. These characteristics can be a selection criterion for the physiological quality of chili seeds.

CONCLUSIONS

Morphological information about chili seeds based on seed color, seed shape, and beak prominence on the seeds can distinguish between chili species. Chili species are difficult to distinguish based on the character of 1000 seed weight because each species can have a different weight category. Seed size in this study did not affect the percentage of seed germination. The physiological diversity of chili seeds has wide genetic variability. Genetic factors affect the character of 1000 seed weight. The species *C. pubescens* has the most different seed size and physiological characteristics of the four other species tested, while *C. annuum* has many similarities with *C. frutescens*, *C. chinense*, and *C. baccatum*.

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