Heliyon 8 (2022) e10888

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Agromorphological characterization of cacao (*Theobroma cacao* L.) accessions from the germplasm bank of the National Institute of Agrarian Innovation, Peru

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HIGHLIGHTS

- The morphoagronomic characterization of cacao clones resulted in the differentiation of 5 groups.
- Group three presented the largest number of accessions differentiated by quantitative characteristics.
- The productivity indicator was decisive in the formation of the groups.

ARTICLE INFO

Keywords:
Accession
Characterization
Descriptors
Conservation
Germplasm

ABSTRACT

Agromorphological characterization of cacao accessions in Peru is currently an important tool in the conservation and genetic improvement of cacao germplasm. The objective of this study was to carry out the morphological and agronomic characterization of 113 cacao accessions from the Huarangopampa germplasm bank. Tree, leaf, flower, fruit and seed descriptors were used. The data collected were processed by descriptive statistics using multivariate techniques. Five groups were formed according to similar characteristics. The accessions of group 1 are vigorous trees with an pod index of 19.27 pods/kg of seeds; the groups that presented better differential characteristics were group 2 with erect tree architecture, intermediate vigorousness, purple seed color and pod index of 20.07 pods/kg of seeds and group 3, which had the highest number of accessions with the lowest pod index of 18.77 pods/kg of seeds, besides being vigorous trees and having purple seeds. On the other hand, group 4 presented a particular characteristic of white seed color and high pod indexes with 22.11 pods/kg of seeds. Finally, group 5 accessions were characterized by intermediate tree architecture and vigor with an pod index of 21.3 pods/kg of seeds. The morphoagronomic characterization constitutes a first advance in the identification of cacaos with potential for genetic improvement and advances in the Peruvian chocolate industry.

1. Introduction

Cacao is a product that in recent years has positioned itself in the national and international markets with its new demand trends (Cruz and Cañas, 2018). Cacao (*Theobroma cacao* L.) is a crop of the Malvaceae family (Alverson et al., 1999), which grows under shade and sustains the chocolate industry (Ramírez-Guillermo et al., 2018). Cacao is a tropical crop grown at latitudes between 10°N and 10°S of the equator (Arvelo et al., 2017).

Cacao is grown in hot and humid regions in more than 50 countries in the continents of Africa, America, Asia and Oceania. In the Americas, there are 23 countries producing cacao for commercial purposes, which makes this a crop of great economic and social importance for the territories where it is produced (Arvelo et al., 2017). In the Americas, countries such as Bolivia, Brazil, Peru, Colombia and Ecuador stand out for their production levels (Batista, 2009). Peru exports between 50 and 75% of its production, corresponding to 7% of international production, which may seem insignificant, but its potential lies in the fact that its

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https://doi.org/10.1016/j.heliyon.2022.e10888
Received 15 March 2022; Received in revised form 27 May 2022; Accepted 28 September 2022

exports are of fine and aromatic native cacao, an added value that allows it to differentiate itself from its competitors (López et al., 2020).

Peru has a high genetic diversity and variability of cacao, present throughout the high and low jungles of the country (Ministerio de Agricultura y Riego, 2016). This native genetic richness, together with the existing soil and climatic characteristics in Peru, provide a competitive advantage in the short and medium term (García, 2008). Knowledge and dissemination of the morphological and agronomic characteristics of the cacao varieties grown in the country and their rescue, conservation and genetic improvement are key aspects for the good management and use of available genetic resources (Ramírez-Guillermo et al., 2018).

The study of morphological aspects of plant species is used to investigate microscopic and macroscopic aspects of form, structure and reproduction, which do not require scientific training (Pérez-García and Mendoza, 2002). Morphological characterization has been and is used as a tool to carry out numerous studies in genetic improvement in agriculture (Arciniegas, 2005). In addition, the conservation of the different accessions is important for genetic improvement programs, for which phenotypic and genotypic characterization is important (Ramos and Gómez, 2019).

In some departments of Peru, cacao accessions were characterized using morphological descriptors of flower, fruit, leaf, seed, production, sensory analysis and molecular analysis, in order to strengthen the use of germplasm through pre-improvement. Along these lines, Oliva (2020) carried out an identified and characterized of the biological diversity of 146 accessions of fine aroma native cacao based on morphological characteristics of fruits and seeds, sensory and productivity descriptors, achieving the characterization of 5 groups for the departments of Amazonas, San Martin and Cajamarca. On the other hand, García (2019) found seven groups, where group four was the most concentrated and

Figure 1. Location of the germplasm bank.

diverse phenotypically in characterizing the 46 cacao accessions held in the international collection of the Universidad Nacional Agraria de la Selva.

In this context, the production of clones is part of a strategy for genetic improvement, since it arises from the selection of the best accessions with outstanding characteristics in quality, productivity and adaptability that will directly influence this crop to be productive and generate the expected yields (Quintana et al., 2015).

The National Institute for Agrarian Innovation has a cacao germplasm bank, located in Huarangopampa annex, which was installed in 2016, given the variability of accessions it has, it is necessary to perform the characterization for future research in genetic improvement, so the objective of this study was to perform the morphological and agronomic characterization of cacao accessions.

2. Materials and methods

2.1. Study area

The germplasm bank of the 113 cacao accessions is located in the annex of Huarangopampa, district of El Milagro, Utcubamba province, located at 552 m a.s.l (Figure 1). The soils of this population center are of a sandy clay loam texture, with pH 7.5, electrical conductivity 4 mS/m, 7.35 ppm phosphorus, 219.81 ppm potassium, 1.09 % organic carbon, 1.88% organic matter and 0.09 % nitrogen, according to results obtained from the soil characterization analysis conducted at the Soil and Water Research Laboratory of the Toribio Rodriguez de Mendoza National University. The climatic characteristics of the area where the germplasm bank was installed are presented in Table 1.

2.2. Plant material

The study was conducted with 113 cacao accessions established in 2016 collected from two provinces, Bagua and Utcubamba. Bagua is characterized by two types of climates: warm (14.5 °C–25 °C, with rainfall of 500–4000 mm) and is located between 500 and 3500 m a. s. l. (meters above sea level). It has mountainous landscapes of the mountainous and hilly eastern range of the sub-Andean Mountain range, flat and hilly and alluvial plains of the Marañon, Utcubamba and tributaries, and arid warm climate, which includes the areas of lower altitude of the province, with average annual temperatures of 25. 1 °C, with an average annual rainfall of 1,400 mm and altitudes ranging from 350 to 1,400 m a. s. l. It includes the following landscapes: mountainous, hilly, undulating

Table 1. Climatic variables of the study area for the year 2021.

Months	Climatic variables		
	Temperature	Accumulated precipitation (mm)	Relative humidity (%)
January	23.25	30.3	77.28
February	24.11	0.9	74.54
March	22.74	125.2	82.42
April	23.08	62.4	82.19
May	22.95	141.1	80.76
June	22.58	32.3	80.13
July	22.41	8.5	73.38
August	23.71	12.2	69.88
September	23.57	63.7	73.75
October	24.06	131.1	77.31
November	24.17	34.1	75.46
December	24.21	43.2	75.68

Source: Bagua automatic weather station (Servicio Nacional de Meteorología e Hidrología del Perú, 2021).

Table 2. Geographical location of the 113 cacao accessions.

N° of accession	District	Locality	Latitude	Longitude	Altitude
1	Copallin	Caña Brava	5°45′8.86″	78°25′17.09″	1152
2	Copallin	Caña Brava	5°45′10.72″	78°25′16.04″	1152
3	Copallin	Caña Brava	5°38′52.22″	78°24′33.82″	1063
4	La Peca	Humbete	5°37′18.32″	78°25′0.87″	1119
5	La Peca	Humbete	5°37′18.28″	78°25′0.57″	1121
6	La Peca	Humbete	5°36′21.99″	78°25′6.34″	1055
7	La Peca	Humbete	5°36′54.59″	78°25′6.78″	1054
8	La Peca	Humbete	5°36′54.53″	78°25′8.44″	1055
9	La Peca	Humbete	5°36′54.63″	78°25′8.34″	1055
10	La Peca	Humbete	5°36′44.94″	78°25′8.35″	1052
11	La Peca	Arrayan	5°36′1.22″	78°26′31.68″	898
12	La Peca	San Francisco	5°37′27.95″	78°26′59.07″	727
13	La Peca	San Francisco	5°37′27.97″	78°27′5.01″	710
14	El Parco	El Parco	5°37′37.21″	78°28′11.55″	614
15	El Parco	El Parco	5°37′37.18″	78°28′11.68″	615
16	El Parco	El Parco	5°37′36.99″	78°28′12.55″	612
17	El Parco	El Parco	5°37′47.39″	78°28′11.70″	614
18	El Parco	El Parco	5°37′36.62″	78°28′11.06″	614
19	El Parco	Tolopampa	5°36′1.19″	78°29′3.37″	629
20	Bagua	El Tomaque	5°39′29.61″	78°29′53.24″	469
21	Bagua	El Tomaque	5°39′29.61″	78°29′53.24	469
22	Bagua	El Tomaque	5°39′28.53″	78°29′51.54″	469
23	Bagua	El Tomaque	5°39′27.61″	78°29′52.35″	469
24	Copallín	Huaranguillo	5°40′39.18″	78°27′3.65″	443
25	Copallín	Huaranguillo	5°40′38.76″	78°27′4.40″	443
26	Copallín	Huaranguillo	5°40′39.57″	78°27′5.71″	449
27	Copallín	Huaranguillo	5°40′39.61″	78°27′5.50″	449
28	Copallín	Copallín	5°40′28.41″	78°24′38.33″	948
29	Copallín	Copallín	5°40′28.94″	78°24′39.17″	944
30	Copallín	Copallín	5°40′29.14″	78°24′40.73″	936
31	Copallín	Copallín	5°40′27.01″	78°24′38.53″	943
32	Copallín	Lluhuana	5°40′34.78″	78°24′29.17″	932
33	Copallín	Lluhuana	5°40′56.43″	78°24′17.90″	908
34	Copallín	Lluhuana	5°40′58.02″	78°24′17.47″	894
35	Copallín	Lluhuana	5°40′58.35″	78°24′17.28″	894
36	Copallín	Lluhuana	5°40′58.66″	78°24′15.46″	899
37	Copallín	Lluhuana	5°41′1.50″	78°24′17.36″	887
38	Copallín	Lluhuana	5°40′57.62″	78°24′21.24″	896
39	Copallín	Lluhuana	5°40′55.07″	78°24′19.40″	900
40	Copallín	Lluhuana	5°40′46.82″	78°24′14.86″	920
41	Copallín	La Palma	5°39′11.83″	78°23′18.78″	1177
42	Copallín	La Palma	5°39′7.60″	78°23′18.76″	1182
43	Copallín	La Palma	5°39′8.01″	78°23′16.52″	1173
44	Copallín	Lluhuana	5°41′2.54″	78°24′8.72″	832
45	Copallín	Lluhuana	5°41′1.11″	78°24′9.47″	864
46	Cajaruro	San Jossé Bajo	5°42′7.94″	78°24′3.10″	740
47	Cajaruro	San Jossé Bajo	5°42′7.87″	78°24′3.07″	739
48	Cajaruro	San Jossé Bajo	5°42′5.78″	78°24′7.23″	735
49	Cajaruro	San Jossé Bajo	5°42′5.78″	78°24′7.17″	737
50	Cajaruro	San Jossé Bajo	5°42′6.10″	78°24′6.74″	735
51	Cajaruro	San Jossé Bajo	5°42′7.17″	78°24′6.28″	734
52	Cajaruro	San Jossé Bajo	5°42′7.17″	78°24′6.15″	736

(*continued on next page*)

Table 2 (*continued*)

N° of accession	District	Locality	Latitude	Longitude	Altitude
53	Cajaruro	San Jossé Bajo	5°42′6.33″	78°24′7.03″	738
54	Cajaruro	San Jossé Bajo	5°42′7.34″	78°24′7.19″	731
55	Cajaruro	San Jossé Bajo	5°42′8.22″	78°24′7.03″	733
56	Cajaruro	San Jossé Bajo	5°42′7.34″	78°24′7.19″	727
57	Cajaruro	San Jossé Bajo	5°42′5.43″	78°24′1.61″	746
58	Copallin	El Yuyo	5°39′32.70″	78°24′56.24″	944
59	Cajaruro	El Manantial	5°44′20.73″	78°20′56.61″	720
60	Copallín	Lluhuana	5°41′2.00″	78°27′28.79″	900
61	Copallín	Pan De Azucar	5°40′2.65″	78°23′13.32″	1039
62	Copallín	Pan De Azucar	5°40′0.59″	78°23′10.34″	1044
63	Copallín	Pan De Azucar	5°40′1.18″	78°23′10.44″	1043
64	Copallín	Pan De Azucar	5°40′1.50″	78°23′10.53″	1044
65	Cajaruro	Diamante Bajo	5°43′14.82″	78°20′55.68″	808
66	Cajaruro	Diamante Bajo	5°43′11.91″	78°20′53.94″	832
67	Cajaruro	Diamante Bajo	5°43′10.80″	78°20′53.10″	851
68	Cajaruro	Diamante Bajo	5°43′32.37″	78°20′52.35″	553
69	Cajaruro	Diamante Bajo	5°42′42.93″	78°19′50.61″	856
70	Cajaruro	Diamante Bajo	5°42′41.35″	78°19′48.41″	846
71	Cajaruro	Diamante Bajo	5°42′40.90″	78°19′48.19″	845
72	Cajaruro	Diamante Bajo	5°42′40.93″	78°19′47.83″	845
73	Cajaruro	Diamante Bajo	5°42′42.93″	78°19′50.61″	844
74	Cajaruro	Diamante Bajo	5°42′41.42″	78°19′47.57″	840
75	Cajaruro	Diamante Bajo	5°42′41.61″	78°19′47.76″	842
76	Cajaruro	Diamante Bajo	5°42′41.48″	78°19′47.57″	840
77	Cajaruro	Diamante Bajo	5°42′39.56″	78°19′48.29″	843
78	Cajaruro	Diamante Bajo	5°42′38.85″	78°19′49.59″	853
79	Imaza	Pakun	5°10′23.46″	78°16′51.78″	278
80	Imaza	Pakun	5°10′23.30″	78°16′51.81″	278
81	Imaza	Pakun	5°10′26.58″	78°16′50.27″	276
82	Imaza	Pakun	5°10′26.58″	78°16′59.33″	283
83	Imaza	Duran	5°13′34.59″	78°21′11.90″	346
84	Cumba	Cumba	5°56′14.11″	78°39′33.88″	540
85	Cumba	Cumba	5°56′13.18″	78°39′31.16″	476
86	Cumba	Cumba	5°57′3.77″	78°38′48.50″	496
87	Cumba	Cumba	5°57′4.25″	78°38′48.01″	503
88	Cumba	Cumba	5°57′4.94″	78°38′48.20″	496
89	Cumba	Arcana	5°57′1.35″	78°39′25.75″	474
90	Cumba	Arcana	5°57′56.56″	78°38′36.87″	461
91	Cumba	Arcana	5°57′20.02″	78°38′36.34″	463
92	El Milagro	Cayaltí	5°46′11.88″	78°34′45.49″	647
93	El Milagro	Cayaltí	5°46′9.15″	78°34′46.48″	643
94	El Milagro	Cayaltí	5°46′5.74″	78°34′46.91″	647
95	Bagua Grande	Jahuanga	5°46′21.30″	78°33′24.36″	614
96	Bagua Grande	Jahuanga	5°46′21.36″	78°33′24.52″	614
97	Bagua Grande	Jahuanga	5°46′21.20″	78°33′24.82″	614
98	Bagua Grande	Jahuanga	5°46′37.01″	78°33′30.96″	629
99	Bagua Grande	Jahuanga	5°46′37.66″	78°33′31.80″	630
100	Bagua Grande	Jahuanga	5°46′38.55″	78°33′32.61″	632
101	Bagua Grande	Jahuanga	5°46′39.22″	78°33′31.50″	629
102	Bagua Grande	Jahuanga	5°46′39.22″	78°33′31.50″	629
103	Copallín	Lluhuana	5°40′56.27″	78°24′17.97″	894
104	Copallín	Lluhuana	5°40′56.44″	78°24′19.17″	891
105	Bagua	Peca Palacios	5°40′0.21″	78°29′28.39″	491
106	Imaza	Shushunga	5°12′5.11″	78°19′52.67″	362
107	Imaza	Shushunga	5°12′5.11″	78°19′52.67″	362
108	Cajaruro	La Cruz	5°45′31.92″	78°24′45.51″	454
109	Cajaruro	La Concordia	5°44′9.56″	78°24′40.58″	496
110	Cajaruro	Misquiaco Bajo	5°44′55.92″	78°20′33.19″	786
111	Cajaruro	La Concordia	5°44′8.24″	78°24′37.54″	517
112	Cajaruro	La Concordia	5°44′9.58″	78°24′38.60″	517
113	Cajaruro	La Concordia	5°44′9.33″	78°24′40.78″	497

plains (sub-Andean Mountain range) and the plains of the Marañón and Utcubamba rivers. Rainfall is 600–800 mm per year (Ministerio de Agricultura y Riego, 2008).

The province of Utcubamba has a varied climate. In the lower parts of the territory, it has a warm climate (up to 40 °C, with an average rainfall of 1,300 mm per year). In areas with altitudes between 400 and 1,400 m a. s. l., the predominant landscapes are: Mountainous, hilly, undulating flat and the Marañon and Utcubamba river plains. At altitudes between 1400 and 2900 m a. s. l., the climate is temperate with temperatures ranging between 14 and 25 °C and rainfall ranging from 500 to 3500 mm per year. In this area, the following landscapes predominate: mountainous of the Eastern Cordillera, mountainous and hilly of the Sub-Andean Cordillera, flat and hilly, and the alluvial plains of the Marañón, Utcubamba and tributaries rivers (Flor et al., 2013).

The cacao plants that made up the germplasm bank were collected on June 14, 2016. A total of 113 accessions (of national provenance) were selected from a total of 122, of which 9 corresponded to an international provenance so they were already characterized and were not selected for the study. The collection sites were georeferenced and are presented in Table 2 and Figure 2.

The cacao accessions were collected as budwood for subsequent asexual propagation by scion grafting. The budwood came from parent plants in production that were six years old (Gárate et al., 2020).

2.3. Experiment design

The design consisted of 9 plants per cacao accessions (replicates), which were established with a distance of 3 m between plants and 3 m between rows, using a square layout (Quiroz and Mestanza, 2012). The germplasm bank was installed on June 15, 2016 and the evaluations for

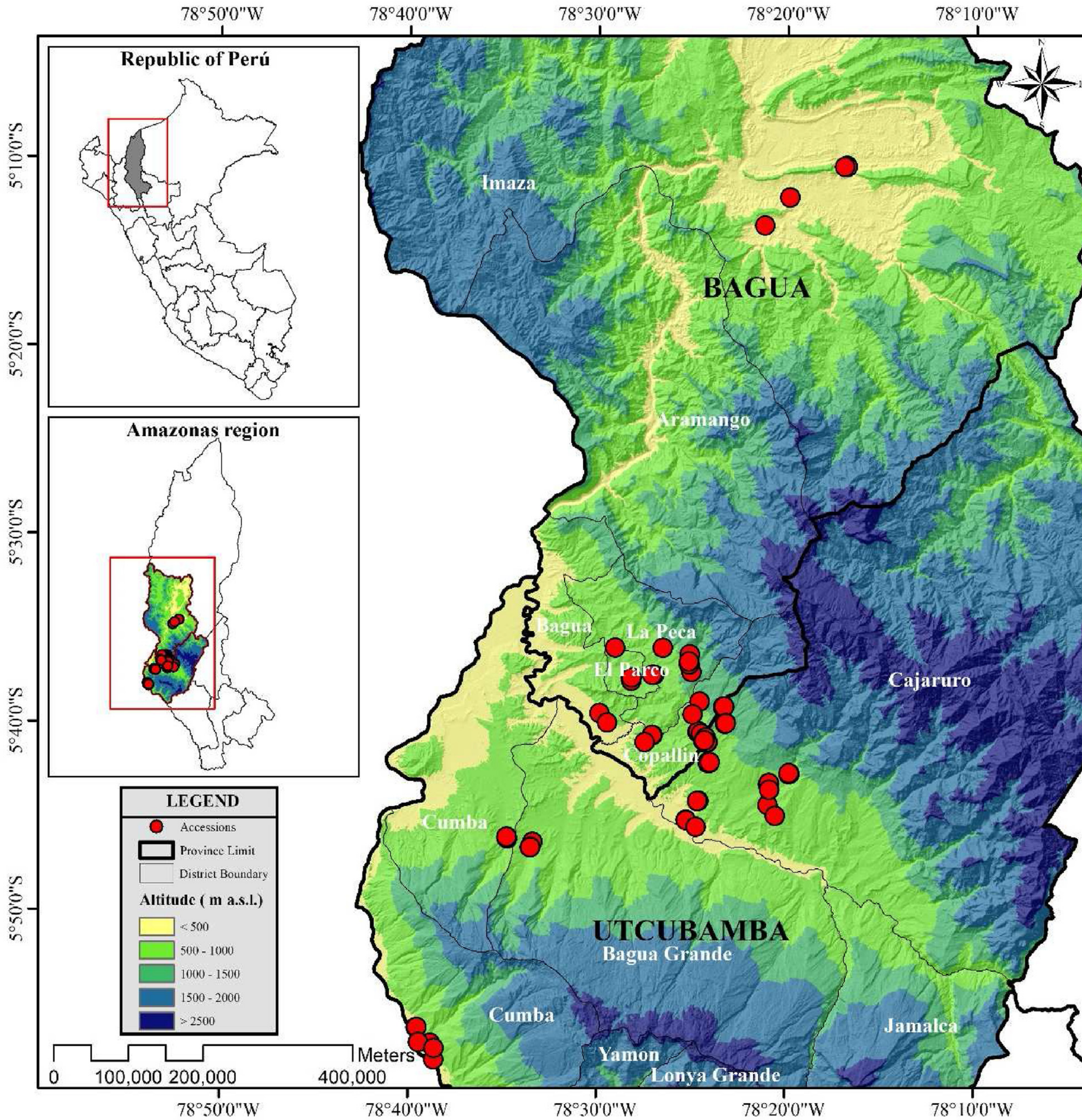

Figure 2. Location of cacao accessions sampling points.

characterization were carried out from February to December 2021 when the accessions were 5 years old.

2.4. Morphological and agronomic descriptors

The descriptors were worked under the methodology of López et al. (2018), García (2010) and Restrepo and Urrego (2018) considering tree, leaf, flower, fruit and seed descriptors of 113 cacao accessions (Table 3, Figure 3A–H).

2.5. Agronomic descriptors

The agronomic descriptors were for leaf: leaf length (cm), leaf width (cm), petiole length (cm), for flower: pedicel length (cm), sepal length (mm), sepal width (mm), petal length (mm), petal width (mm), filament length (mm), staminate length (mm), style length (mm), ovary length (mm), ovary width (mm) and seed: number of furrows, fruit weight (g), fruit length (cm), fruit diameter (cm), shell thickness (cm), furrow depth (cm), pericarp weight (g), number of locules, number of seeds per locule, seed fresh weight (g), number of seeds per fruit, number of whole seeds, number of empty seeds, weight of 10 seeds (g), seed length, seed width, seed thickness, pod index (Phillips-Mora et al., 2012; Restrepo and Urrego, 2018; Soto, 2019).

2.6. Statistical analysis

For the characterization of the 113 cacao accessions, they were analyzed using multivariate techniques with the use of InfoStat/Professional Software version 2018p (Di Rienzo et al., 2008). A cluster analysis was applied using Ward's method, Gower's distance, in order to group

Table 3. Morphological descriptors.

Descriptor	Indicator
Tree architecture	1 = Erect, 2 = Intermediate, 3 = Perpendicular
Tree vigor	1 = Weak, 2 = Intermediate, 3 = Vigorous
Branch formation	1 = Simple, 2 = Intermediate, 3 = Verticillated
Leaf base shape	1 = Sharp, 2 = Obtuse, 3 = Round, 4 = Cordiform
Leaf apex shape	1 = Accumulated, 2 = Short sprinkled, 3 = Long sprinkled, 4 = Warm
Color of tender bud	1 = Light green, 2 = Medium green, 3 = Brown, 4 = Light red, 5 = Medium red, 6 = Dark red
Color of adult leaf	1 = Light green, 2 = Medium green, Dark green
Presence of anthocyanins in pedicel	1 = Absent, 2 = Weak, 3 = Moderate, 4 = Strong
Anthocyanin pigment of sepals	1 = Absent, 2 = Weak, 3 = Moderate, 4 = Strong
Pigment of staminodes	1 = Absent, 2 = Weak (Slight) 3 = Moderate, 4 = Strong
Ovary external color	1 = Cream, 2 = Yellow, 3 = Creamy yellow 4 = Light green
Color of immature fruit	1 = Light green, 2 = Dark green, 3 = Pigmented green, 4 = Dark red
Color of ripe fruit	1 = Green Yellow, 2 = Yellow, 3 = Orange, 4 = Medium Red, 5 = Dark Red, 6 = Purple.
Fruit shape	1 = Elliptical, 2 = Oblong, 3 = Bent, 4 = Ovate, 5 = Orbicular, 6 = Oblate
Apex shape	1 = Sharp, 2 = Toothed, 3 = Sharp, 4 = Nipped, 5 = Obtuse, 6 = Rounded
Shape of basal constriction	0 = Absent, 1 = Slight, 2 = Intermediate, 3 = Strong
Fruit roughness	0 = Absent, 1 = Slight, 2 = Intermediate, 3 = Strong
Seed color	1 = White, 2 = Violet, 3 = Purple
Seed shape	1 = Oblong, 2 = Elliptical, 3 = Ovate, 4 = Irregular
Shape of seed in cross section	1 = Flattened, 2 = Intermediate, 3 = Rounded

according to similarity, after which the analysis of variance was performed to determine the characteristic that influences the formation of groups for quantitative variables, using the Fisher LSD test. With the qualitative (categorical) data, contingency tables were made to determine the characteristics that contributed to the formation of groups, and with the variables that were significant, a multiple correspondence analysis was performed to determine the association between the qualitative variables evaluated.

3. Results

3.1. Characterization of groups of clones within the germplasm

The characterization was carried out by cluster analysis, using Ward's method and Gower's distance for the leaf, flower, fruit and seed descriptors, and five groups of cacaos were identified (Figure 4).

The analysis of variance of the groups formed for all the variables evaluated showed no significant statistical differences, with the exception of the pod index ($p = 0.04$), which was a determining variable for the formation of the five groups.

Group 4 had the highest mean for this variable. Three accessions were found with 14 pods/kg of seeds, six accessions with 15 pods/kg of seeds and 15 accessions with 16 pods/kg of seeds. This productivity indicator showed significant statistical differences between groups indicating that it was a determinant variable in the formation of groups within the quantitative characteristics ($p = 0.04$), where the highest pod indices were found in groups 4 and 5 (Figure 4, Table 4) with 22. 11 ± 4.10 and 21.3 ± 5.11 pods/kg seeds respectively, intermediate values with groups 2 and 1 with 20.07 ± 3.76 and 19.27 ± 4.17 respectively and the lowest pod index was achieved with group 3 with 18.77 ± 3.22 pods/kg seeds.

Group 1 is formed by 11 accessions (Figure 4) showing the highest averages for staminode length (6.91 ± 1.07), ovary width (1.28 ± 0.26), fruit weight (857.64 ± 316.71), fruit length (20.27 ± 4. 08), fruit diameter (20.27 ± 4.08), pericarp weight (650.91 ± 268.28), number of locules (5 ± 0.00), number of seeds (7.91 ± 1.45), fresh weight of seeds (187.64 ± 54.14), number of seeds per fruit (40.36 ± 4.65) (Table 4).

Group 2 consists of a set of 30 cacao accessions (Figure 4), the highest values were found for characteristics such as leaf width (11.32 ± 2.09), petiole length (2.46 ± 0.65), shell thickness (1.73 ± 0.37) and seed thickness (1.03 ± 0.20) (Table 4).

Figure 4 shows that group three has the highest number of accessions with 31 cacao beans, the higher values correspond to sepal width (2.62 ± 1.14), ovary length (1.76 ± 0.39), furrow depth (1.28 ± 0.48), seed length (4.39 ± 9.03) and seed width (1.48 ± 0.26) (Table 4).

Group 4 is represented by the conformation of 18 cacao (Figure 4), and Table 4 shows the highest mean values for characteristics such as leaf length (33.96 ± 5.65), petiole length (17. 63 ± 4.31), sepal length (8.18 ± 1.32 a), petal length (3.74 ± 0.42), filament length (5.54 ± 0.83), number of seeds per pod (2.44 ± 2.59), weight of 10 seeds (44.17 ± 14.87). Group 5 consists of 23 cacao beans (Figure 4), the highest averages are presented for the following characteristics of petal width (1.82 ± 0.33), style length (2.98 ± 0.83) and number of whole seeds (35.13 ± 9.23) (Table 4).

3.2. Morphological characteristics

Contingency tables were used to determine the association between the categorical variables of tree, leaf, flower, fruit and seed descriptors (Table 5). It was found that 18 of 20 variables evaluated were significant for the separation of 5 groups.

3.3. Multiple correspondence analysis (MCA) for tree descriptor

The first two axes explained 30.85% variation base on MCA and showed that group two was associated with erect tree architecture and simple branches, whereas group 4 consisted of weaker tree and group 5 characterized by intermediate tree architecture and vigor (Figure 5).

3.4. Leaf descriptors

The first two axes explained 36.07 % of the variation based on the MCA and showed that groups 2 and 3 were associated with a light green adult leaf color and tender shoot color ranging from light green to medium green, while groups 1 and 4 were characterized by presenting dark green adult leaf color and brown tender shoot color. Group 5 was mainly characterized by the round shape of the leaf base (Figure 6).

3.5. Flower descriptors

The first two axis of the MCA explained 20.54% of the variation and showed that group 3 and 5 were associated with moderate anthocyanin

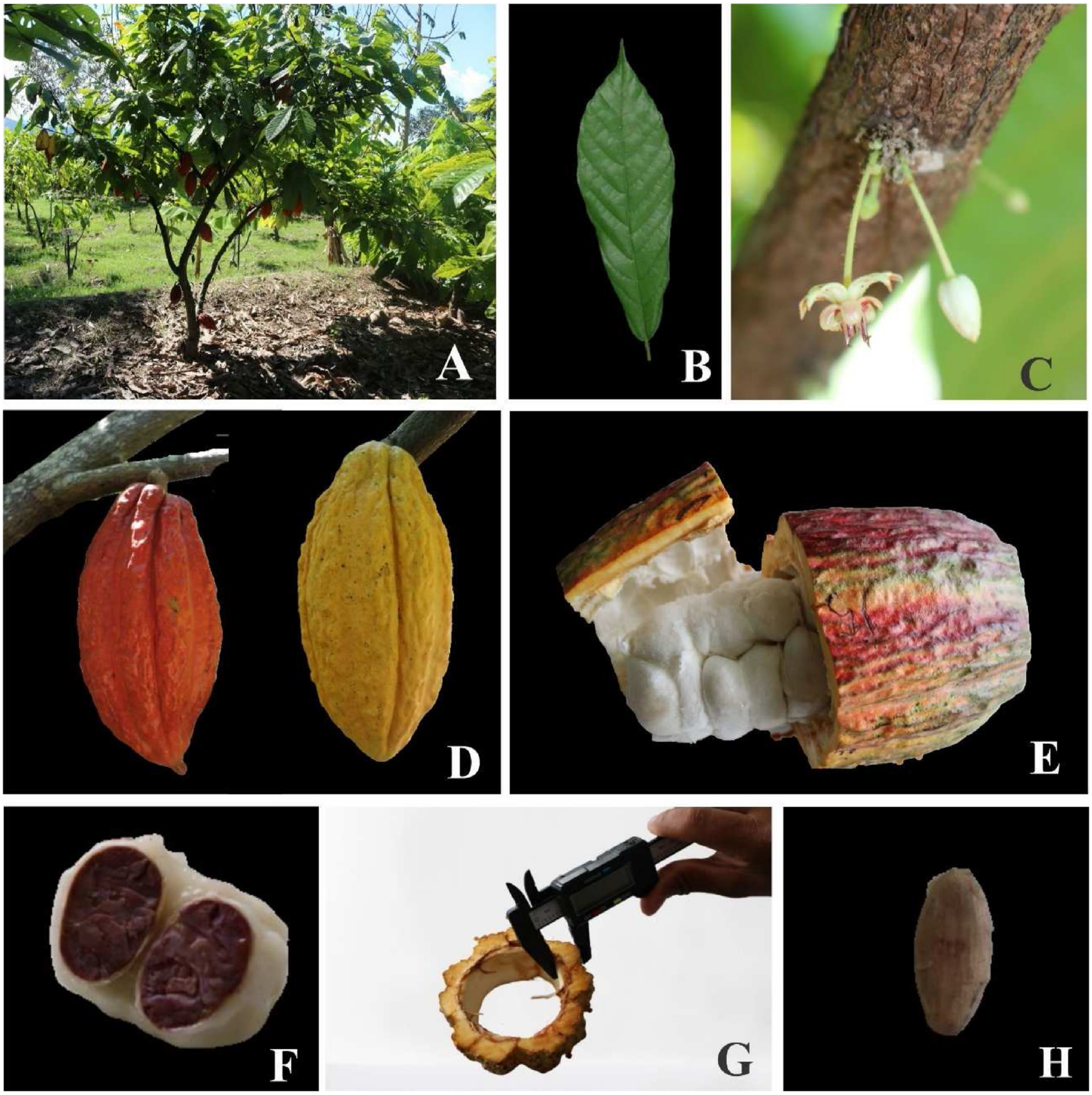

Figure 3. Descriptors: tree (A), leaf (B), flower (C), fruit (D, E and G) and seed (F and H).

in the pedicel and cream ovary color. Group 2 is characterized by the absence of anthocyanins in the sepals (Figure 7).

3.6. Fruit descriptor

Figure 8 shows the MCA explained a cumulative percentage of 19.84 % for its first two axes with group three being associated with absence of basal constriction shape, the fruits present an elliptical shape with obtuse apices. Group 2 is characterized by yellow ripe fruit color, attenuated apex shape, light basal constriction shape. Group 5 is mainly characterized by oblong fruits with acute apex and intermediate basal constriction.

3.7. Seed descriptor

MCA showed (Figure 9) that the first two axis explained 30.47% of the variation (Figure 9) where group three was characterized by violet seeds and irregular shape while group 4 consisted of mainly white seeds and group 2 oval shaped seeds of a purple color and a round cross section.

In general, the accessions of group 1 were vigorous with a medium green color of tender shoots and had an average pod index of 19.27 pods/kg seeds; group 2 accessions were the accessions with more differentiated characteristics, the tree architecture was erect, with intermediate vigor, simple branch formation, short leaf apex shape, light green adult leaf color, no anthocyanins in the sepals, strong pigmentation of staminodes, yellow color of immature fruits, attenuated acute apex shape, light basal constriction shape, purple seed color, oval longitudinal seed shape, round transverse seed shape, and intermediate pod index of 20.07 pods/kg seed (Table 6). Group 3 accessions were vigorous, with short leaf apex shape, light green adult leaf color, moderate presence of anthocyanins in the pedicel, strong pigmentation of staminodes, elliptical fruit shape, absent basal constriction shape,

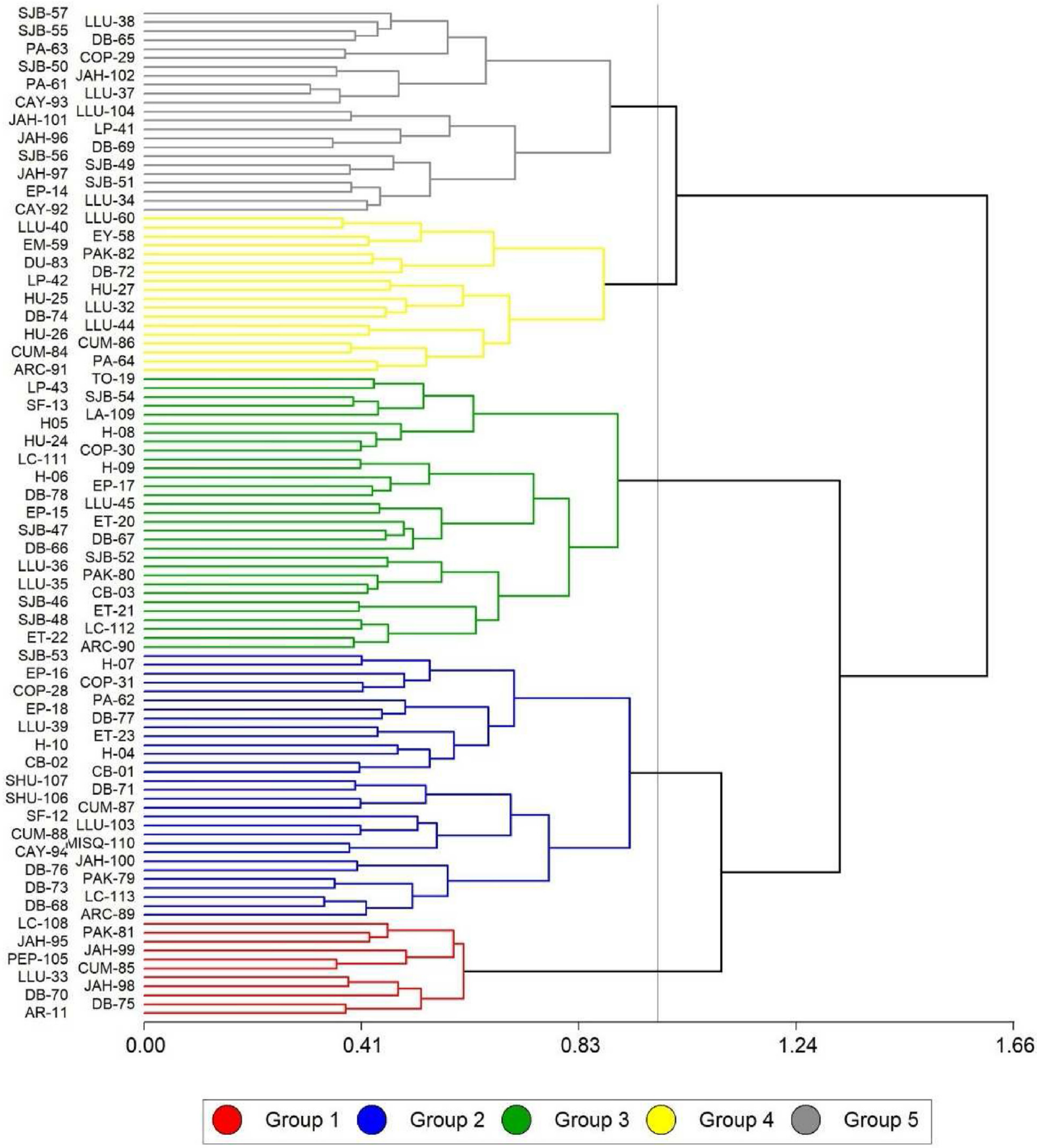

Figure 4. Dendogram resulting from cluster analysis (Ward's method 0.99:5 groups and Gower's distance for 113 accessions according to morphological and agronomic characteristics.

violet seed color, irregular longitudinal seed shape, and had a low pod index (18.77 pods/kg seed) (Table 6). On the other hand, group 4 accessions, had weak tree vigor, brown color of tender shoot, dark green color of adult leaf, white seed color and present high pod index with 22.11 pods/kg seeds. Finally, group 5 accessions, had intermediate tree architecture and vigor, round leaf shape, short leaf apex shape, moderate presence of anthocyanins in the pedicel, strong staminode pigmentation, cream ovary external color, oblong fruit shape, acute apex shape, intermediate basal constriction shape and pod index of 21.3 pods/kg seeds (Table 6).

4. Discussion

The characterization of cacao accessions has allowed differentiating five groups of cacao clones, grouped according to their quantitative and qualitative characteristics, thus demonstrating the high genetic variability and heterogeneity among groups based on the ordering of tree, leaf, flower, fruit and seed descriptors (López-Hernández et al., 2019). The conformation of the groups was similar to that reported by Oliva (2020), who found 5 differentiated groups out of a total of 146 accessions evaluated in three regions of Peru (Amazonas, Cajamarca and San

Table 4. Analysis of variance for quantitative data for leaf, flower, fruit and seed descriptors for 5 cacao groups.

G	Leaf length	Leaf width	Petiole length	Pedicel length	Sepal length	Sepal width	Petal length	Petal width	Filament length	Staminate length
	F = 1.20	F = 0.08	F = 0.12	F = 0.99	F = 1.04	F = 0.96	F = 0.95	F = 1.66	F = 1.46	F = 2.33
	P = 0.32	P = 0.99	P = 0.98	P = 0.4181	P = 0.39	P = 0.46	P = 0.4364	P = 0.1636	P = 0.22	P = 0.06
1	31.85 ± 3.42	11.26 ± 1.51	2.34 ± 0.25	16.08 ± 1.33	7.38 ± 1.10	2.36 ± 0.56	3.71 ± 0.71	1.65 ± 0.23	5.21 ± 0.1	6.91 ± 1.07
2	31.93 ± 5.01	11.32 ± 2.09	2.46 ± 0.65	16.19 ± 2.72	7.46 ± 1.71	2.32 ± 0.53	3.49 ± 0.52	1.66 ± 0.33	5.03 ± 0.83	6.33 ± 1.11
3	32.01 ± 4.19	11.19 ± 1.51	2.39 ± 0.52	16.19 ± 2.73	7.50 ± 1.43	2.62 ± 1.14	3.50 ± 0.60	1.79 ± 0.25	4.93 ± 0.90	6.13 ± 0.97
4	33.96 ± 5.65	11.19 ± 1.67	2.40 ± 0.52	17.63 ± 4.31	8.18 ± 1.32	2.29 ± 0.42	3.74 ± 0.42	1.81 ± 0.31	5.54 ± 0.83	6.82 ± 1.04
5	30.94 ± 3.16	11.07 ± 1.01	2.41 ± 0.67	17.09 ± 3.46	7.87 ± 1.32	2.30 ± 0.55	3.57 ± 0.46	1.82 ± 0.33	5.08 ± 1.00	6.72 ± 0.97

G	Stylar length	Length of ovary	Ovary width	Number of furrows	Fruit weight	Fruit length	Fruit diameter	Peel thickness	Furrow depth	Pericarp weight
	F = 0.26	F = 0.48	F = 0.70	F = 0.68	F = 1.60	F = 0.60	F = 1.17	F = 1.69	F = 1.60	F = 0.97
	P = 0.90	P = 0.75	P = 0.5940	P = 0.60	P = 0.18	P = 0.66	P = 0.33	P = 0.16	P = 0.18	P = 0.43
1	2.89 ± 0.38	1.75 ± 0.21	1.28 ± 0.26	5 ± 0.00	857.64 ± 316.71	20.27 ± 4.08	10.05 ± 1.17	1.73 ± 0.37	1.10 ± 0.17	650.91 ± 268.28
2	2.78 ± 0.63	1.74 ± 0.31	1.24 ± 0.26	5 ± 0.00	703.43 ± 241.36	19.68 ± 3.78	9.34 ± 1.27	1.92 ± 0.54	1.22 ± 0.29	546.47 ± 201.38
3	2.84 ± 1.02	1.76 ± 0.39	1.20 ± 0.22	5 ± 0.00	672.74 ± 179.09	18.72 ± 3.10	9.40 ± 1.01	1.74 ± 0.53	1.28 ± 0.48	533.65 ± 145.71
4	2.92 ± 0.58	1.74 ± 0.21	1.17 ± 0.13	5 ± 0.00	702.28 ± 247.05	19.11 ± 3.04	9.34 ± 0.98	1.77 ± 0.46	1.10 ± 0.23	434.56 ± 201.50
5	2.98 ± 0.83	1.65 ± 0.24	1.17 ± 0.26	5 ± 0.00	654.78 ± 223.56	19.61 ± 3.09	9.20 ± 1.10	1.56 ± 0.46	1.07 ± 0.38	518.52 ± 193.63

G	Number of locules	Number of seeds/locule	Fresh weight of seeds	Number of seeds/fruit	Number of whole seeds	Number of empty seeds	Weight of 10 seeds	Seed length	Seed width	Seed thickness
	F = 2.00	F = 0.11	F = 1.30	F = 1.29	F = 1.58	F = 0.88	F = 0.41	F = 0.81	F = 0.44	F = 0.59
	P = 0.09	P = 0.0.98	P = 0.27	P = 0.29	P = 1.18	P = 0.49	P = 0.79	P = 0.52	P = 0.78	P = 0.67
1	5 ± 0.00 a	7.91 ± 1.45	187.64 ± 54.14	40.36 ± 4.65	38.91 ± 4.50	1.45 ± 1.44	43.82 ± 12.69	2.67 ± 0.43	1.45 ± 0.28	1.03 ± 0.20
2	4.97 ± 0.18	7.83 ± 1.39	162.07 ± 53.04	36.77 ± 8.84	35 ± 9.60	1.50 ± 2.37	41.23 ± 10.61	2.53 ± 0.37	1.43 ± 0.25	1.04 ± 0.12
3	4.97 ± 0.18	7.71 ± 1.44	185.87 ± 54.07	40.26 ± 7.07	38.87 ± 7.52	1.39 ± 1.58	41 ± 13.38	4.39 ± 9.03	1.48 ± 0.26	1 ± 0.15
4	4.83 ± 0.38	7.83 ± 1.10	177.56 ± 84.86	36.39 ± 9.13	33.94 ± 9.23	2.44 ± 2.59	44.17 ± 14.87	2.67 ± 0.48	1.44 ± 0.32	1.02 ± 0.17
5	4.78 ± 0.52	7.65 ± 1.50	155.65 ± 51.48	36.70 ± 9.41	35.13 ± 9.23	1.57 ± 1.88	40.09 ± 8.91	2.56 ± 0.43	1.37 ± 0.34	0.98 ± 0.11

G: Group.

Table 5. Contingency tables for the associativity of morphological descriptors for group formation.

Tree descriptor	Leaf descriptor	Flower descriptor	Fruit descriptor	Seed descriptor
Tree architecture*	Leaf shape*	Presence of anthocyanins in the pedicel**	Fruit color Immature^{ns}	Seed color**
Tree vigor**	Leaf apex shape**	Presence of anthocyanins in sepals**	Color of ripe fruit**	Seed shape (Longitudinal cut)**
Branch formation*	Tender shoot color**	Pigment in staminodes*	Fruit shape**	Seed shape (cross section)**
	Adult leaf color**	Ovary external color**	Apex shape**	
			Basal constriction shape**	
			Fruit roughness^{ns}	

**: Highly significant, *: Significant and ns: not significant.

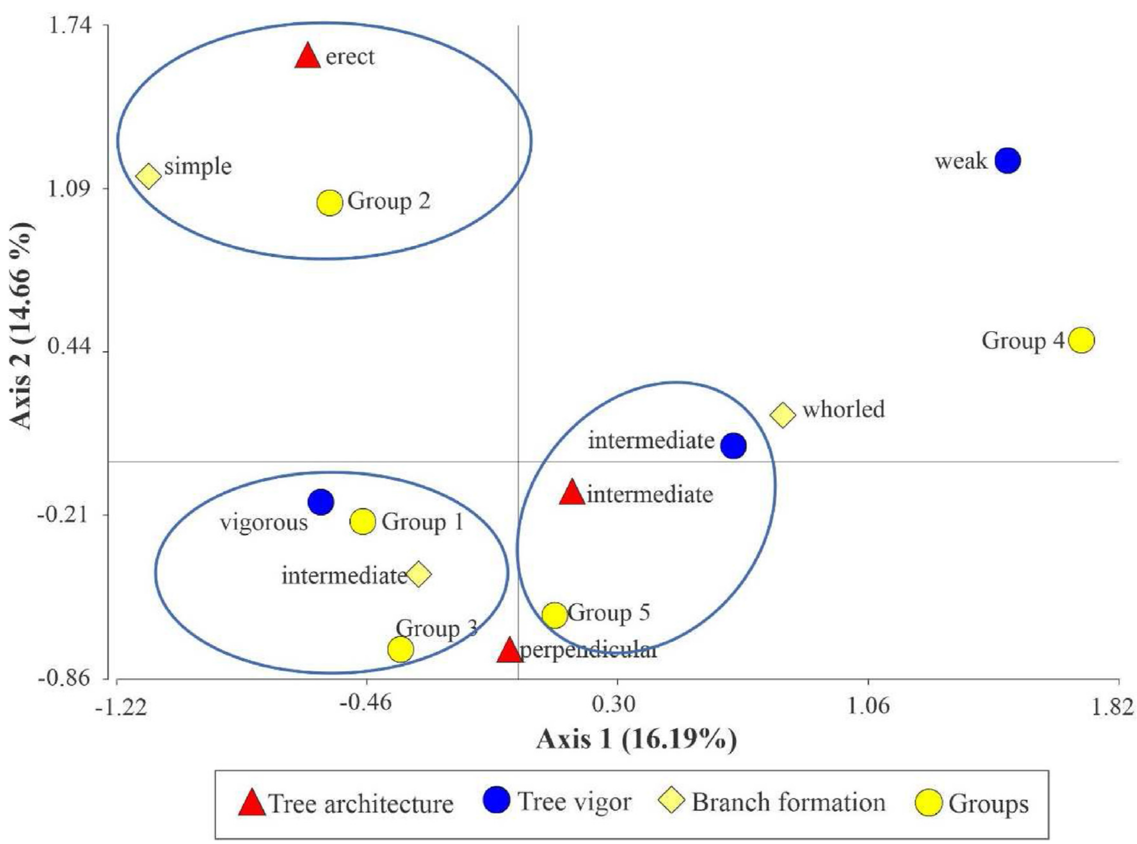

Figure 5. Multiple correspondence analysis of groups formed for tree descriptor (architecture: erect, intermediate and perpendicular; vigor: weak, intermediate and vigorous and branch formation: simple, intermediate and whorled).

Martin), based on morphological descriptors of fruits and seeds, sensory characteristics and productivity descriptors. On the other hand, Quevedo et al. (2020a) found different results using the same cluster analysis and Ward's method, reporting 10 groups formed for 650 cacao accessions in their morphoagronomic characterization study in Ecuador. The conformation of clusters is due to the genetic variability of the accessions studied, the greater the variability, the greater the number of clusters formed (López-Hernández et al., 2019).

Fruit related characteristics such as weight, length and diameter were superior to those reported by Martínez (2016), who found an average weight of 272.6 g, length between 11.5 and 16.6 cm and diameter between 7 and 8.1 cm, in his study of genetic variability of Bolivian National cacao.

Group 1 presented the highest average (40.36 ± 4.65) of seeds per fruit, being lower than those reported by Franco-Portillo et al. (2019), who found a maximum of 47 seeds per fruit for two accessions of Criollo cacao in El Salvador, this will depend on the amount of fertilized ovules (Rangel-Fajardo et al., 2012).

The observable morphological or physiological characteristics of a species or population depend on the genotype and this can be influenced by environmental and nutritional factors (Pérez and Freile, 2017; Botero and Arias, 2018). Group three of this research was mainly characterized by vigorous trees with intermediate branches, which could be due to good agronomic management (Ayestas et al., 2013; López et al., 2018).

In the evaluation of qualitative variables, 90% were significant for the formation of groups. Villegas and Astorga (2005) found that these variables are the ones that best explain the variability between groups. Groups two and three presented light green leaf color, this is due to the absence of anthocyanin content (Gutiérrez, 2020). In addition, it was found that the presence of anthocyanins in the staminodes was significant in the formation of the groups, this is explained by its discriminant value and taxonomic importance as indicated by Restrepo and Urrego (2018). This presence of anthocyanins, could be due to the fact that they correspond to accessions of national origin and others of foreign origin from the Upper Amazon (García, 2019).

The shape of abovate fruit was the second least representative within the 113 accessions characterized, this characteristic is uncommon in germplasm collections in Peru, and could be attributed to a private allele and the highest percentage corresponds to the shape of oblong fruit, García

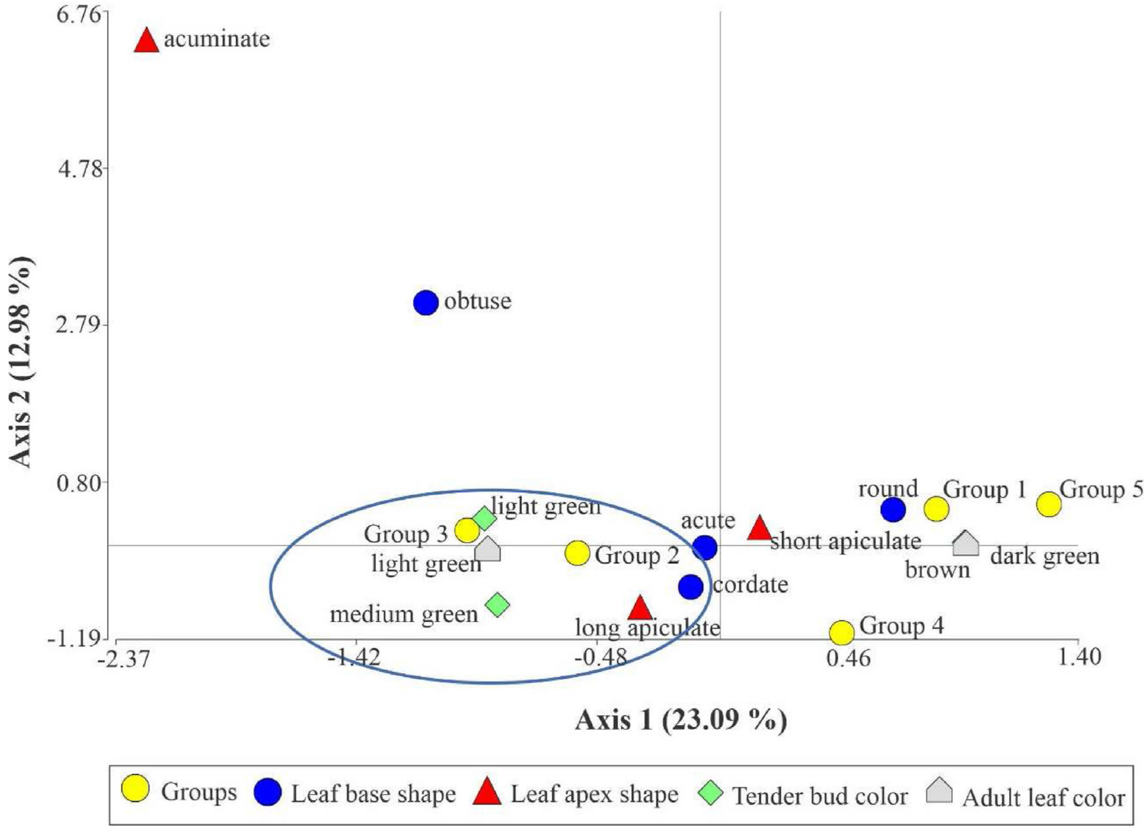

Figure 6. Multiple correspondence analysis of groups formed for leaf descriptor (shape of leaf base: acute, obtuse, round, cordate; shape of leaf apex: acuminate, short apiculate, long apiculate; color of tender shoot: light green, medium green and brown; odor of adult leaf: light green and dark green).

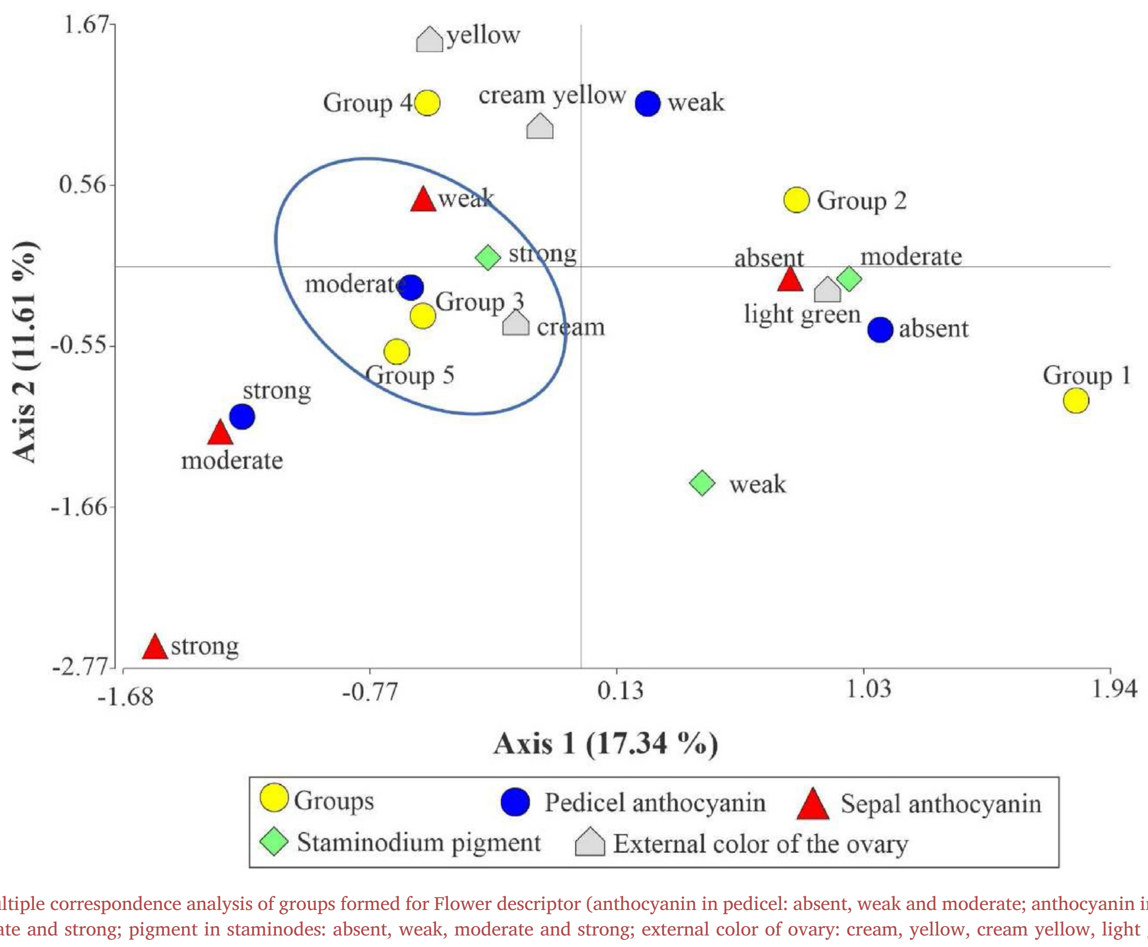

Figure 7. Multiple correspondence analysis of groups formed for Flower descriptor (anthocyanin in pedicel: absent, weak and moderate; anthocyanin in sepal: absent, weak, moderate and strong; pigment in staminodes: absent, weak, moderate and strong; external color of ovary: cream, yellow, cream yellow, light green).

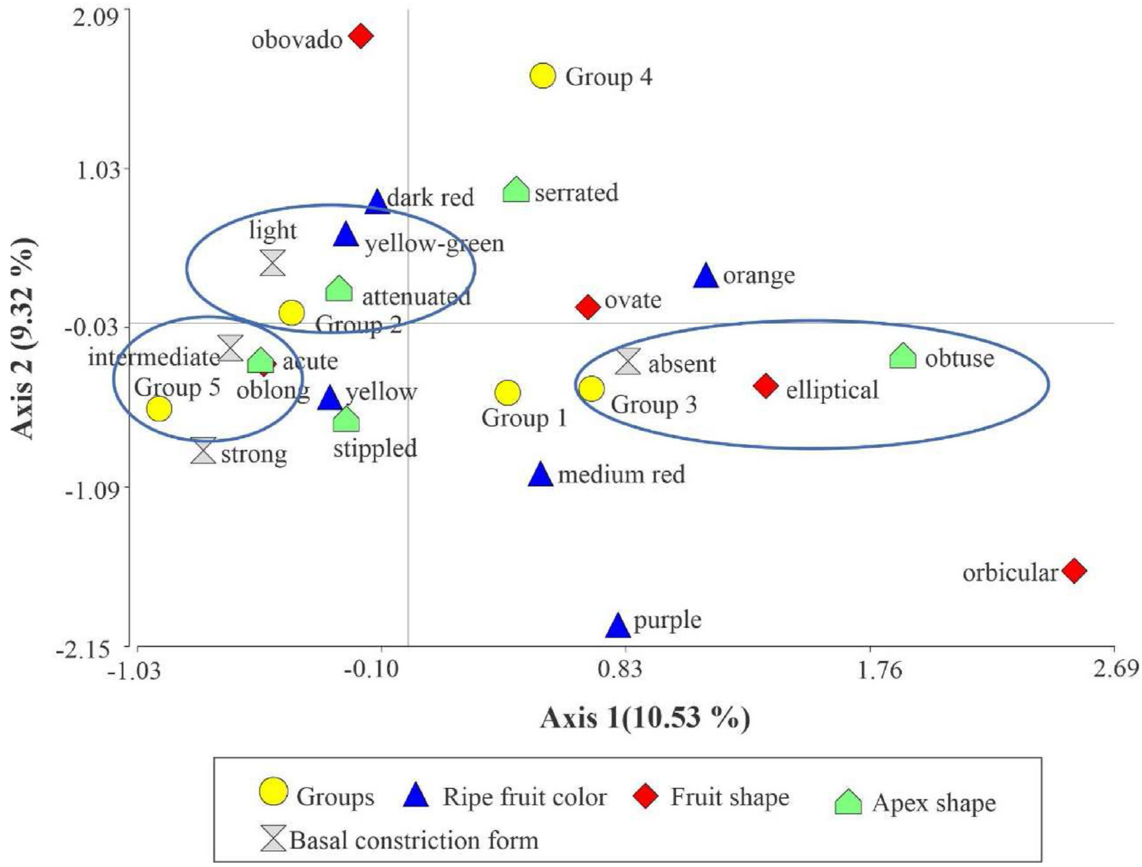

Figure 8. Multiple correspondence analysis of groups formed for fruit descriptor (color of ripe fruit: yellow green, yellow, orange, medium red, dark red and purple; shape of fruit: oblong, obovate, ovate and orbicular; shape of apex: attenuated, serrated, acute, serrated; shape of basal constriction: absent, light, intermediate and strong).

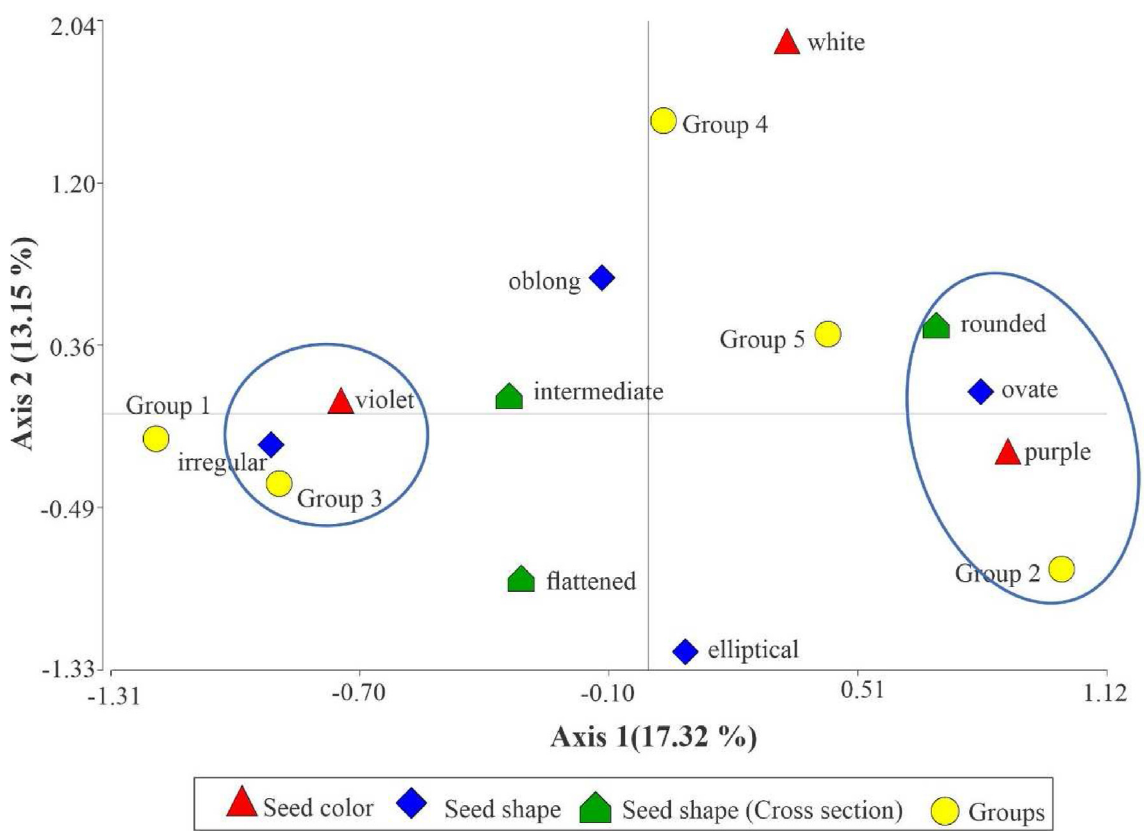

Figure 9. Multiple correspondence analysis of groups formed for seed descriptor (seed color: white, violet and purple; longitudinal cut seed shape: oblong, elliptic, ovate; transverse cut seed shape: flattened, intermediate and rounded).

Table 6. Differential morphoagronomic characteristics of 113 cacao accessions.

Variables	Group 1	Group 2	Group 3	Group 4	Group 5
Tree architecture		Erect			Intermediate
Tree vigor	Vigorous	Intermediate	Vigorous	Weak	Intermediate
Branch formation		Simple			
Leaf shape					Round
Leaf apex shape		Short	Short		Short
Color of tender bud	Medium green			Brown	
Adult leaf color		Light green	Light green	Dark green	
Presence of anthocyanins in pedicel			Moderate		Moderate
Presence of anthocyanins in sepals		Absent			
Pigment in staminodes		Strong	Strong		Strong
Ovary external color			Cream		Cream
Color of immature fruit		Yellow			
Fruit shape			Elliptical		Oblong
Apex shape		Attenuated			Acute
Shape of basal constriction		Slight	Absent		Intermediate
Seed color		Purple	Violet	White	
Longitudinal seed shape		Ovate	Irregular		
Transverse seed shape		Round			
Pod index	19.27 ab	20.07 ab	18.77 b	22.11 a	21.3 a

(2019) mentions that in interclonal crosses of cultivars 'Forastero del Alto Amazonas' with the cultivar: H-12, this shape is the most frequent.

The characteristics of the fruit with respect to the roughness of the fruit were not significant in the formation of groups, this characteristic could be associated to the hybridization of native × forastero cacao from the lower Amazon (Ramírez-Guillermo et al., 2018). The variability of basal constriction presented in the cacao groups formed may be due to the fact that they correspond to genetic groups coming from Trinitario or Amazonian forastero cacao (Bartra, 2009).

Group four was characterized by presenting white seeds, being an important characteristic due to the preference for chocolates from cotyledons of these colors that are associated with quality (Ramírez-Guillermo et al., 2018). In the present study, a diversity of shapes and colors was found in the mature and immature stages, as a result of cross fertilization of cacao beans (Graziani et al., 2002). Morphological characterization is used to evaluate the phenotypic variability between different cacao accessions since qualitative characters are highly discriminatory and highly heritable, which facilitates the differentiation of accessions (García and García, 2017).

With respect to the pod index, it was found that group three presents an acceptable pod index with 18 cacao pods to produce one kilogram of dry cacao, these results are higher than those obtained by Oliva-Cruz et al. (2021) who found pod indexes between 14 and 16 pods/kg of seeds. On the other hand, the results reported in this research are similar to those reported by Quintana et al. (2015), who found pods indices for ICS 60 clones followed by CCN 51 of 16 and 20 pods per kg.

On the other hand, in the characterization it was found that the 5 groups presented an pod index below 23 pods/kg of seeds, these results were lower than those reported by Graziani et al. (2002), who found an pod index higher than 23 with Criollo, Amazonian forasteros and Trinitario cacaos from Cumboto in Venezuela.

The pod index indicates the productivity of the cacao crop, and is therefore an important characteristic for discriminating cacao accessions and represents an attribute that can be used in the characterization of cacao germplasm (Quevedo et al., 2020b; Motamayor et al., 2002). In this context, it is considered important to take into account the accessions found in group 3 (31 accessions) because they have the lowest pod indices. In addition, it is recommended to complement this study with research at the molecular level, for a better understanding of the genetic relationship of the accessions studied and thus implement breeding strategies for the development and release of new improved varieties (Mustiga et al., 2018) with good quality potential and optimal yield through sustainable production systems (Olasupo and Aikpokpodion, 2019).

5. Conclusion

Cluster analysis allowed classification into five groups showing a high intraspecific variability, which indicates the existence of accessions that differ among them. The greatest number of accessions were grouped in group 3 and can be considered important for productive indicators such as ear index. On the other hand, this group, like group 2, presents accessions with significant morphoagronomic characteristics that help differentiation. Group 4, in spite of having a high pod index, has a characteristic that gives it added value, the presence of white seeds, and can be considered for the chocolate industry. This should be complemented with sensory and molecular characterization of these accessions for a better selection of accessions and subsequent development of genetic improvement.

Declarations

Author contribution statement

Seyed Mohammad Hosseini: Performed the experiments.

Hassan Yousefnia: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Behrouz Alirezapour; Ali Hossein Rezayan; Javad Mohammadnejad: Conceived and designed the experiments; Analyzed and interpreted the data.

Funding statement

Jheiner Vásquez García was supported by INSTITUTO NACIONAL DE INNOVACIÓN AGRARIA (Proyecto de Inversión: "Mejoramiento de los Servicios de Investigación en la Caracterización de los Recursos Genéticos de la Agrobiodiversidad en 17 departamentos del Perú-PROAGROBIO" con CUI 2480490).

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors of this scientific work are grateful to the Investment Project "Mejoramiento de los Servicios de Investigación en la Caracterización de los Recursos Genéticos de la Agrobiodiversidad en 17 departamentos del Perú – PROAGROBIO" with CUI 2480490 of the Instituto Nacional de Innovación Agraria (INIA) for the funding provided to carry out the research at the Amazonas -Huarangopampa Agrarian Experimental Station.

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