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Agromorphological characterization of nine local high-yield varieties of chili (*Capsicum* spp) produced in Central and South Benin

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Abstract

Pepper (*Capsicum* spp.), one of the most important spices and vegetable crops worldwide is considered part of the human diet. This study aim to analyze the genetic resources of local chili varieties for future improvement of their agronomic performance. A total of (9) nine local varieties produced in five different departments were identified and selected from available collection at the Laboratory of Plant Physiology and Environmental Stress Studies. Thus, a Fisher block and three-repetition design was installed on the experimental site. Based on the analyses carried out, it is found that the diameter at the collar (DiaC) was positively and strongly correlated with the height of the plant (HP), the number of branches of the stem (NBS), and the density of the foliage (DFol). Analysis of variance showed no significant difference ($p < 0.05$) between varieties, in terms of a number of branches (NBS), length of most developed branch (LenB), span of foliage (SFol) and foliage density (DFol). However, the varieties 'Fundja', 'Gbatakin', 'Takin gaga', and 'Tchobo' showed a long germination time, smaller diameter and height of plants, leaf area, number of branches, lengths of the most developed branch, foliage spans, and average foliage densities.

Key words: *Capsicum* spp, genetic diversity, vegetable, morphology, yield

Introduction

Pepper (*Capsicum* spp.), one of the most important species of the Solanaceae family, is grown and consumed worldwide (Moon et al., 2023; Duranova et al., 2022; Ledi et al., 2020). According to Sampaio et al., (2023), its global production is 38.3 million tons on a cultivated area of 19 million hectares in 2021. The three largest pepper producers are China (45.3%), Mexico (8.4%) and Turkey (6.3%) (Sampaio et al., 2023). It is a fruit vegetable grown throughout the world and of vital importance for human consumption (Dias et al., 2013). Originating in South and Central America, it has spread throughout Europe, Africa and Asia. In Benin, after the tomato, the chili is the most requested condiment in urban environments for consumption because of its spicy flavor. It is cultivated both in rainy and off-season (Biao et al., 2019) and is consumed in fresh, dry, whole or powder form (McCoy et al., 2023). The chili fruits are rich in ascorbic acid, carotenoids, phenolic compounds and flavonoids (Ben Abed et al., 2022; Lahbib et al., 2021; Zhuang et al., 2012). Numerous studies have demonstrated the high nutritional value of chili, its health benefits and its medicinal properties (Batiha et al., 2020; Moon et al., 2023; Saleh et al., 2018). Most chili species are diploid with 24 chromosomes (2n = 24). Among the genus Capsicum, which harbors more than 35 species, 5 species (*C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*) are domesticated (Bosland et al. 2012). These species produce fruits of different shapes, colors and sizes; these fruits may have different degrees of pungency, which is determined by the concentration of capsaicinoids specific to this gene (DeWitt and Bosland 2009; Moscone et al., 2006; Sampaio et al., 2023). The genetic variability of a crop favors the development of new cultivars with differences in shape, color and yield of the fruits. Maintaining diversity is therefore important for its application as a crop improvement program tool (Sampaio et al., 2023). Preserving and conserving this variability in germplasm banks and in situ conditions allows the selection of genotypes that are valuable in different market niches, which in turn favors agro-industry and family farming. Several studies conducted in different countries of the world have shown the importance of knowing and studying the diversity of a species in order to better explore genetic variability in reproduction, conservation and promote its use for the benefit of humans (Cardoso et al., 2018; Gu et al., 2019; Sampaio et al., 2023).

The objective of this study is to analyze the genetic resource of nine local chili available in order to contribute to the improvement of the agronomic performance of this species.

Material and Methods

Presentation of the study area

The municipality of Abomey-Calavi is located in the Atlantic department of the Republic of Benin. It lies between the parallels 6° 22' and 6° 30' of North latitude and the meridians 2° 15' and 2° 22' of East longitude (Figure 1). It is bounded in the north by the commune of Zè, in the south by the Atlantic Ocean, in the east by the commune of Cotonou and So-Ava and in the west by the communes of Ouidah and Tori-Bossito. The municipality has nine (9) districts: Abomey-Calavi; Akassato; Godomey; Golo-Djigbé; Hèvié; Kpanroun; Ouèdo; Togba; Zinvé and one hundred and forty-nine (149) villages and city districts. (SDG, 2019). It covers an area of 539 km^2; almost 20% of the area of the department and 0.47% of the actual national area, and has a population estimated at around 1,191,620 inhabitants in 2023 with a rate of increase of 6.7% with 570,073 women and 553,547 men (INStaD, 2023). The municipality of Abomey-Calavi enjoys a sub-equatorial climate characterized by four seasons: a large rainy season (April to July); a small rainy season (September to November); a large dry season (December to March); and a small dry season (August to September). The rainfall of the municipality is 1200 mm/year and breaks down as follows: 700 mm to 800 mm for the large season and 400 mm to 500 mm for the small season (Tonon, 2007).

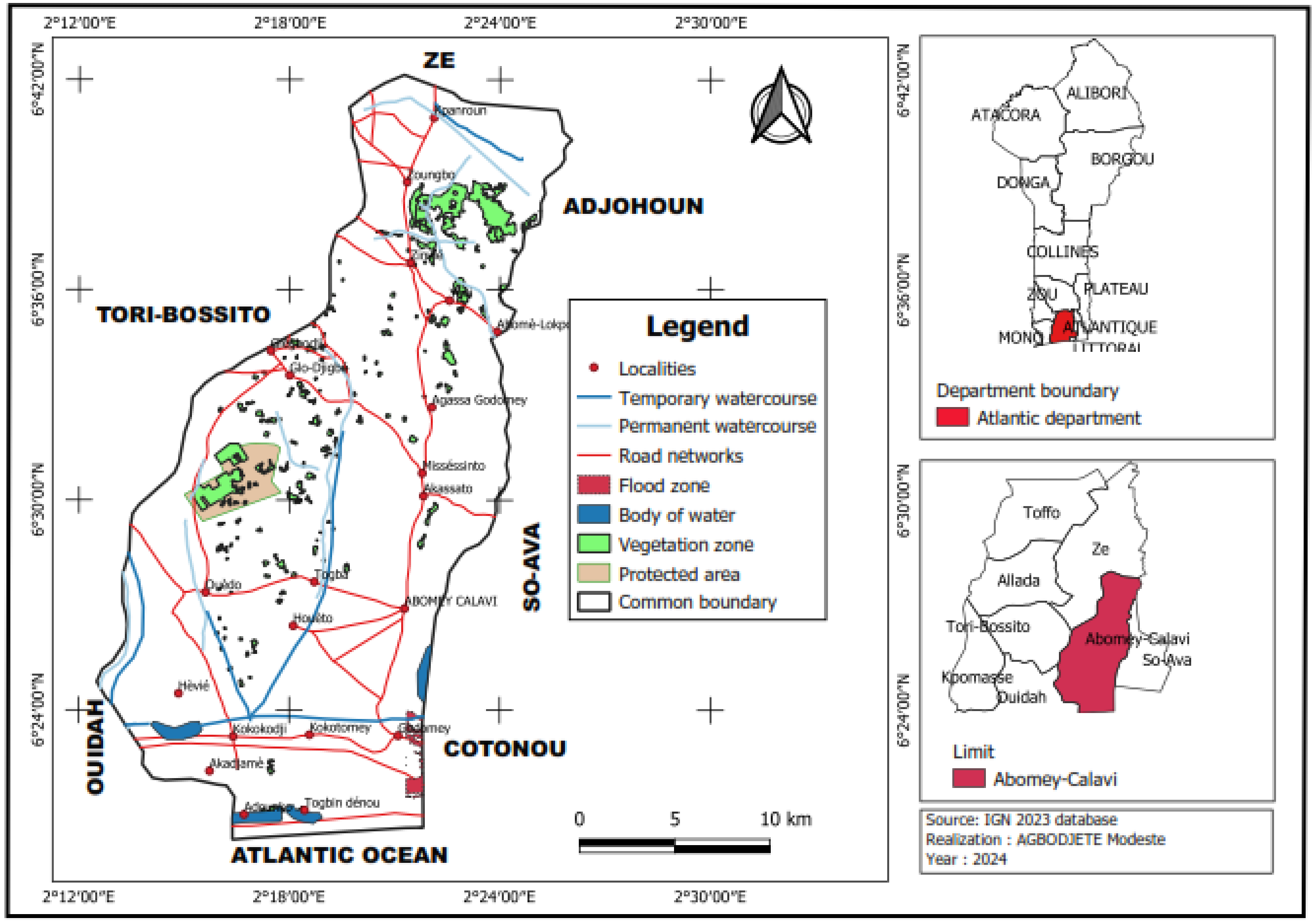

Figure 1 : Administrative map of Abomey-Calavi

Study material

The study material will consist of 9 local varieties of pepper from the collection made by Hounyeme in 2023 (Unpublished data) and kept at the Laboratory of Plant Physiology and Environmental Stress Study of the University of Abomey-Calavi. Three species, *Capsicum annum*, *C. frutescens,* and *C. chinense* constitute the different groups of peppers produced in the study area.

Methods

Experimental design

The seeds of each variety, after having been selected following a flotation test to verify the viability of the germs, were sown in a nursery and straw. Four (04) to five (05) days after the seedlings were raised, an 80 cm tall shade was constructed using palm branches. Daily watering with plain water was provided. The shade was removed gradually from the 15th day after sowing until it was completely removed seven days before subculturing.

For the implementation of the experiment, the elementary plot consists of a seedling line. Thus, 27 plots 7.5 m long were demarcated on a cleared plot. The spacing between these plots was 1 m. The experimental plot is then 9 m wide and 28.5 m long, i.e. an area of 256.5 m^2.

Seedlings' transferring from the nursery

Vigorous seedlings with at least 4 to 5 true leaves selected at random from the nursery were transplanted to the experimental plots, 45 days after sowing, following a Fisher block device (Dagnelie, 2000) consisting of nine treatments in three repetitions. The blocks were separated by 1 m. Each block contained 9 experimental units representing, at random, each of the nine varieties. Each experimental unit carried 15 plants at 0.5 m intervals, i.e. 135 plants per block and 405 plants on the three blocks of the test. Daily watering (morning and evening) with ordinary water and manual weed control, once every two weeks, were carried out until the end of the experiment to ensure good growth and development of the organs of the plant material

Data collection

The data collected concerned the germination time (Dger) and the morphological parameters recorded at the flowering of the plants. These variables are neck diameter (DiaC), plant height

(HP), number of main stem branches (NBS), most developed branch length (LenB), foliage span (SFol), leaf area (SurFo), and foliage density (DFol). Density is evaluated on a scale from 1 to 3 and described as follows: 1 = low density, 2 = medium density, and 3 = high density.

Data Analysis

The data collected were subjected to descriptive and multivariate statistical analyses using Statistica version 7.1 software. They were previously processed using the Excel 2019 spreadsheet. The Pearson linear correlations between the variables were calculated. The data collected was subjected to an analysis of variance. The General Linear Model (GLM) was used to test the variety effect on all eight variables at 5%. In the case where the analysis was significant, homogeneous grouping of the means of the values obtained by variable and by variety was carried out by the Tukey HSD test at the threshold of 5%. A standardized, centered-reduced Principal Component Analysis (PCA) were performed to demonstrate the structure of the variety diversity of chili peppers.

Results

Characteristics of the varieties studied and correlation between the variables

The nine chili varieties used and studied are distributed according to Table 1 and selected from the results of the Hounyeme work in 2023 (Unpublished data) on the collection of local chili varieties produced in the south and the center of Benin.

Table 1: Elite varieties of chili pepper found in the different production areas surveyed

Num	Varieties	Code	Departments (Municipalities)	Cash
01	Elysée	CO1	Hills (Ouesse)	*C. frutescens*
02	Gbatakin	CO2	Hills (Glazoue)	*C. chinense*
03	Hêtakin	CO3	Hills (Djidja)	*C. annuum*
04	Adologbo	CL1	Couffo (Lalo)	*C. frutescens*
05	Takin gaga	ZO1	Zou (Cove)	*C. frutescens*
06	Tchobo	ZO2	Zou (Ouinhi)	*C. frutescens*
07	Fundja	OR1	Ouémé (Adjohoun)	*C. annuum*

08	Tchokossou	AT2	Atlantic (Toffo)	*C. frutescens*
09	Danmetakin	PL1	Tray (Ifangni)	*C. annuum*

The analysis of the diversity of elite local varieties of chili peppers in studies was based on the evaluation of their morphological characteristics. Analysis of the correlation matrix indicates a strong correlation between some pairs of variables. Thus, a strong, significantly positive correlation is obtained between the diameter at the collar (DiaC) and the variables branching number (NBS), plant height (HP), and foliage density (DFol). Furthermore, the foliage density (DFol) is strongly and positively correlated with the variables HP, NBS, and SFol as presented in Table 2.

Table 2: Correlations between measured variables

Variables	Dger	DiaC	HP	NBS	LenB	SFol	LA	DFol
Dger	1.000							
Diac	-0.288	1.000						
HP	- 0.510	**0.914******	1.000					
NBS	- 0.205	**0.894*****	**0.750****	1.000				
LenB	0.382	0.484	0.186	0.606	1.000			
SFol	- 0.144	0.611	0.645	0.536	0.254	1.000		
LA	0.248	- 0.203	- 0.221	- 0.365	- 0.052	0.459	1.000	
DFol	- 0.363	**0.875*****	**0.822*****	**0.904******	0.521	**0.775****	0.072	1.000

Dger: germination time, Diac: Collar diameter, HP: plant height, NBS: number of branches, LenB: most developed branch length, SFol: foliage span, LA: leaf area, DFol: foliage density. In bold, significant at 5%. **: moderate, ***: high, ****: very high.

Variability of the different characters during the study

In order to identify traits that could be useful for future variety improvement, the number of branches (NBS), the length of the most developed branch (LenB), the spread of the foliage (SFol) and the density of the foliage (DFol) showed no significant difference between the varieties following the analysis of variance carried out at the 5% threshold (Table 3). Based on the germination time, the significance test indicates that elite varieties can be subdivided into 4 groups. These are the group of very late varieties represented by 'Fundja' (8.58 days); the group of late varieties represented by 'Elysée' (7.46 days), the group of early varieties 'Tchokossou', 'Danmtakin', 'Takin gaga' and 'Gbatakin' and the group of very early varieties subdivided into 2 subgroups including 'Hetakin', and 'Tchobo and Adologbo' (Table 3). Three groups are derived from plant height analysis (HP), including the variety 'Adologbo' with the highest value (23.02 cm); 'Hêtakin' with intermediate height (19.82 cm), and the remaining seven varieties with lower plant height with values between 14.43 cm and 16.55 cm. For all the varieties studied, greater variability is obtained with the leaf area allowing six different groups to be identified. The varieties "Hêtakin" and "Elysée" with the best average area respectively 20.57 cm^2 and 19.36 cm^2 are unique in their respective group. These varieties are followed by 'Tchokossou' (17.25 cm^2) and 'Danmétakin' (15.02 cm^2) belonging to the same statistical group. Obtained in glazoué, "Gbatakin" has a lower leaf area (13.515 cm2) than the previous, which is unique in its group. Three elite varieties, 'Fundja', 'Takin gaga', and 'Tchobo', have average values between 12.08 and 12.16 cm^2 and form the largest group following this variable. Finally, the 'Adologbo' variety is the only variety with a smaller leaf area with an average value of 11.62 cm^2 (Table 3).

Table 3: Evaluation of the variability between the different characters measured

Varieties	**Dger** *(days)*	**DiaC** (mm)	**HP** *(cm)*	**NBS**	**LenB** *(cm)*	**SFol** *(cm)*	**LA** (cm)	**DFol**
Elysée	7.46±2.32^{ab}	3.613±1.110^{a}	15.31±5.82^{b}	3.13±1.93a	5.11±3.80^{a}	13.02±4.97^{a}	19.36±11.01^{ac}	2.14±0.81^{a}
Gbatakin	6.37±1.56^{abc}	3.475±1.037^{a}	16.55±6.25^{b}	3.35±1.79^{a}	4.45±3.87^{a}	11.69±4.25^{a}	13.515±8.42^{bd}	2.00±0.80^{a}
Hêtakin	6.00±0.62^{bc}	4.028±0.916^{a}	19.82±7.58^{ab}	3.86±2.21^{a}	5.52±4.14^{a}	14.05±3.19^{a}	20.57±14.56^{ab}	2.54±0.74^{a}
Adologbo	5.04±1.51^{c}	4.137±0.659^{a}	23.02±4.41^{a}	3.54±1.53^{a}	4.25±2.29^{a}	11.81±2.53^{a}	11.62±4.39^{cd}	2.27±0.66^{a}
Takin gaga	6.43±2.34^{abc}	3.679±1.004^{a}	15.24±4.43^{b}	3.50±1.87^{a}	5.04±3.51^{a}	10.57±4.16^{a}	12.11±7.15^{bcd}	2.00±0.88^{a}
Tchobo	5.54±1.14^{c}	3.291±0.912^{a}	15.80±5.41^{b}	3.14±1.56^{a}	4.16±3.50^{a}	11.75±4.78^{a}	^{bcd}, 12.08±6.60	2.04±0.84^{a}
Fundja	8.58±1.88^{a}	3,800±1,273^{a}	15.83±6.04^{b}	3.83±1.99^{a}	6.71±4.76^{a}	10.42±4.27^{a}	^{bcd}, 12.16±9.23	2.00±0.95^{a}
Tchokossou	6.86±0.38^{abc}	3.367±0.537^{a}	14.43±3.60^{b}	2.00±1.92^{a}	2.69±2.06^{a}	11.43±2.51^{a}	^{abcd} 17.25±6.96	1.7±0.95^{a}
Danmetakin	6.33±1.92^{abc}	3.370±0.910^{a}	15.41±4.45^{b}	2.67±1.83^{a}	2.83±1.03^{a}	11.42±3.42^{a}	15.02±9.39^{abcd}	1.75±0.74^{a}
F	8.076	2.047	5.670	1.1797	1.5744	1.612	3.9836	1.6616
P	< 0.001	0.041938	< 0.001	0.312074	0.133264	0.122150	< 0.001	0.108671

Dger: germination time, Diac: Collar diameter, HP: plant height, NBS: number of branches, LenB: most developed branch length, EnFron: foliage span, LA: leaf area, DFol: foliage density, mm: millimeter, cm: centimeter, cm^2: square centimeter, F: Fischer test value, p: Fischer test probability.

The mean values in a column followed by the same letter or letters do not differ significantly by 5% according to the Fisher test

Analysis of varietal diversity

Three axes of the main component describe all the variations found during this evaluation of genetic diversity. Thus, the first two axes describe 75.79% of all observed variations while 73.07% of the variabilities are observed considering axes 1 and 3 of the same main component. The diversity of the nine varieties of chili is therefore analyzed in the light of these two plans.

It shows from this analysis that DiaC, HP, NBS, and DFol are very strongly and positively correlated ($0.905 \leq r \leq 0.973$) with axis 1. These variables contributed to the formation of the positive part of Axis 1. Thus, Dger and LA were significantly and negatively correlated ($-0.762 \leq r \leq -0.676$) at axis 2. The Dger has contributed to the negative part of this axis; LA participates to a lesser degree. This LA variable was also negatively and almost moderately correlated ($r = -0.692$) with axis 3. Consequently, four groups of variables were distinguished (Table 4). This correlation between the variables on the different axes allow to describe the constitution of the groups following this analysis. Indeed, the elite local varieties divided into three groups are as follows. The varieties 'Gbatakin', 'Adologbo', 'Tchokossou', and 'Danmétakin' belong to the same group and are well represented on Axis 1; 'Elysée' and 'Tchobo' are better represented on Axis 2; while 'Hêtakin', 'Takin gaga' and 'Fundja' are well positioned on Axis 3 (Table 5).

Table 4 : Correlations between variables and contribution of variables to different axes

Variables	Variable-Axis Correlations			Contributions of variables to axis formation		
	axis 1	axis 2	axis 3	axis 1	axis 2	axis 3
Dger	- 0.545*	- 0.762**	0.407	0.028027	**0.367663**	0.121337
Diac	0.957****	0.025	0.071	**0.204414**	0.000382	0.003659
HP	0.905****	0.254	- 0.166	**0.182645**	0.040965	0.020256
NBS	0.931****	- 0.002	0.293	**0.193284**	0.000002	0.063119
LenB	0.505*	- 0.573*	0.562*	0.056944	**0.207747**	**0.232132**
SFol	0.729**	- 0.383	- 0.528*	0.117109	0.092827	**0.204625**
LA	- 0.168	- 0.676*	- 0.692*	0.006307	**0.287272**	**0.351638**
DFol	0.973****	- 0.070	- 0.066	**0.211270**	0.003143	0.003184

Dger: germination time, Diac: Collar diameter, HP: plant height, NBS: number of branches, LenB: most developed branch length, EnFron: foliage span, LA: leaf area, DFol: foliage density

*: low, **:moderate, ****: very high (very high). The values of the parameters that contributed to the axes are bold,.

Table 5 : Contribution of varieties to the functional axes

Varieties	axis 1	axis 2	axis 3
Elysée	0.40401	**48.13084**	9.96852
Gbatakin	**0.07523**	0.02554	0.04686
Hêtakin	2.87133	3.67286	**8.27856**
Adologbo	**46.99119**	12.10337	4.97513
Takin gaga	0.01431	7.94429	**13.00707**
Tchobo	0.00886	**6.46849**	0.71431
Fundja	0.15161	12.98174	**55.81037**
Tchokossou	**38.57042**	1.01913	6.35175
Danmetakin	**10.91303**	7.65374	0.84741

Whatever the axes of the distribution plan for the varieties, five groups of chili varieties have been identified. Group A is represented by 'Adologbo'. Group B is composed of 'Tchokossou' and 'Danmétakin', and Group C of 'Elysée' and 'Hêtakin'. Group D identified itself as 'Fundja', while Group E (GE) is contained in 'Gbatakin', 'Tchobo', and 'Takin gaga'.

Discussion

Knowledge of genetic diversity is essential for its efficient use in breeding programs. This knowledge involves the description of genetic resources. Phenological and morphological characteristics are the first candidates for this description. A characterization was therefore carried out to determine the phenological and morphological diversity of nine elite local varieties of chili peppers listed from accessions collected in four departments of South Benin. A specific feature of these varieties is their appreciation linked to their good performance in the various producing regions. Some authors in other similar studies have also noted variability in several crop species (Touré et al., 2013; Gbaguidi et al., 2015). The failure to identify a variety from one department to another can be a naming problem linked to the introduction of new varieties by extension programs. Thus, the assessment of varieties may be linked to specific factors of production in the environments surveyed (Orobiyi et al., 2015; 2017). This name variability was also noted in a study conducted on some cultures such as voandzou (Touré et al., 2013) and cowpea (Gbaguidi et al., 2015) where local names are attributed to morphotypes and vary by ethnic group and subgroup (Touré et al., 2013; Gbaguidi et al., 2015). Moreover, an accession of Lalo, without variety name, was called “Adologbo” in this study. This case confirms the fact that there are varieties of chili that remain unknown to the users.

The analysis of variance revealed three to six homogeneous groups of varieties with discriminant variables such as germination time, plant height and leaf area. These results indicate a high phenological and morphological variability among these nine varieties. Similar results have been obtained by many authors in chili peppers (Segnou et al., 2012a, 2012b; Waongo et al., 2021) with the difference that no discrimination was obtained in classifying the different species (Andrade et al., 2020).

The low species specification may be related to the small sample size, and to the experimental conditions of these varieties. This observation was also reported by Ravindran et al., (2015) who reported that genetic variability is low within a small geographic area (Ravindran *et al*., 2005). Indeed, the seeds of the varieties acquired in the different municipalities may come from the same genetic source. Furthermore, chili is one of the crops with high potential variability (Djidji, 1995). According to other reports, this variability could be enhanced by the introduction of diversified varieties from some countries and varietal creations to meet multiple user requirements (Fondio et al., 2015; CNRA, 2019). It is also generated by production practices, including seed exchanges between farmers (Delaunay et al., 2008; Missihoun et al., 2012) and polyvarietal cultivation, which consists of growing several varieties in the same field. This may lead to new genotypes made possible by numerous phenomena such as self-fertilization and natural cross-fertilization in the genus Capsicum (Waongo et al., 2021).

Five groups were identified by principal component analysis (PCA) on the first three axes representing 92.82% of the total variability. Pla (1986) suggested at least 80% of the total variability. The hierarchical ascending classification (CAH) has placed the varieties ‘Fundja’, ‘Gros-Gros’, ‘Tchobo’, and ‘Takin gaga’ in a single group and established four groups, three of which are identical to the groups formed by ACP. These groups of varieties are distinguished by the germination time, the height of the plant, and the leaf area. The numbers of genotype groups or accessions obtained by multivariate methods reflect levels of germplasm variability. They may depend on tools and manpower. Thus, based on vegetative growth parameters such as those used in the present study, namely germination time, stem diameter, plant height, leaf dimensions, canopy width, three to nine groups were obtained with 18 to 192 genotypes or accessions (Segnou et al., 2012b; Ballina-Gómez et al., 2013; Nsabiyera et al., 2013; Andrade et al., 202 0; Bianchi *et al.*, 2020: Waongo *et al.*, 2021). Compared to this work, the nine varieties in this study, divided into four groups, show a high level of variability.

In addition, the analysis revealed three trends of varieties with opposite characteristics. One trend includes seeds that germinated within a short time and produced larger-diameter and taller plants. This is the case for the 'Adologbo' variety, whose characteristics are the most different from those of the other varieties. Another relates to seeds that have germinated within a long period and produced plants of smaller diameters and heights, with leaf surfaces, number of branches, lengths of the most developed branch, foliage spans, and average foliage densities. The varieties 'Fundja', 'Gbatakin', 'Takin gaga' and 'Tchobo' are those with these characteristics. Between these two trends lie intermediate combinations of characteristics. A study by Nsabiyera et al. (2013) on 37 genotypes of Capsicum annum from Uganda and Bianchi et al. (2020) of 56 Capsicum chinense accessions from Brazil and Peru confirms the results obtained in this study. In addition, Nsabiyera et al. (2013) found that local varieties are large in size, stem diameter, and canopy, whereas the opposite is true for improved and introduced exotic varieties. According to these observations, 'Adologbo' variety' could be a local variety, and the 'Gbatakin', 'Takin gaga', and 'Tchobo' varieties could be improved. Despite the environmental influences justifying the usefulness of the agromorphological traits in selection, the diversity observed could result from a variation in the genetic makeup of the varieties (N'dri et al., 2011). There is thus a direct relationship between plant growth and development, leaf area, photosynthesis, flowering, and fruiting (Waongo et al., 2021).

Conclusion

Nine varieties of pepper grown in the central and southern regions of Benin were evaluated for their phenological and morphological diversity. They showed great diversity, especially with characters' germination time, plant height, and leaf area. Moreover, the structuring of this diversity has pitted these varieties against each other. Thus, the 'Adologbo' variety, with the shortest germination time, the highest plant height, the best lateral growth and the highest foliage density, differed most from the others. Also, the varieties 'Fundja', 'Gbatakin', 'Takin gaga' and 'Tchobo' showed a long germination time, smaller diameter and height of plants, leaf area, number of branches, lengths of the most developed branch, foliage spans, and average foliage densities. The varieties 'Elysée', 'Hêtakin', 'Tchokossou' and 'Danmétakin' presented intermediate combinations of parameter values. It follows from these results that the phenological and morphological parameters are useful for the characterization of germplasms.

Authorship

Ekpelikpézé Olivier Symphorien, Faton Manhognon Oscar Euloge, Bonou-Gbo Zaki, Agbodjeté Modeste, and Gnancadja-Andre Léopold Simplice contributed to the study conceptualization and designed the experiments. Faton Manhognon Oscar Euloge, Bonou-Gbo Zaki, Agbodjeté Modeste, contributed to the statistical analysis. Bonou-Gbo Zaki, Ekpelikpézé Olivier Symphorien, Faton Manhognon, and Atindéhou Cynthia wrote the manuscript. Gnancadja-Andre Léopold Simplice, Faton Manhognon Oscar Euloge and Lucie Fanou supervised the entire study. Bonou-Gbo Zaki, Faton Manhognon Oscar Euloge, and Ekpelikpeze Olivier Symphorien reviewed and edited the manuscript. All authors contributed to the manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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