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Tolerance to water stress in different varieties of cowpea (*Vigna unguiculata* L.) in seedling phase

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ABSTRACT: Cowpea has great socioeconomic importance for the Northeast region of Brazil. However, this region suffers from water scarcity, leading to reduced production. Therefore, it is important to identify varieties adapted to water stress conditions. With that aim, this work characterized cowpea creole varieties at the seedling stage using ISSR markers and the Screening Box method. DNA samples extracted from 50 creole varieties were evaluated using ISSR markers. Among them, two were used as controls, one tolerant (Pingo de Puro 1,2) and the other susceptible (Santo Inácio) to drought. Multivariate and cluster analyses were used to identify genetic variability for drought tolerance. Then, an experiment using the Screening Box method was carried out under a greenhouse, where water deficit was induced in 22 varieties, including the control ones. These plants were selected based on the genetic characterization performed with the ISSR. In the first experiment, 45 varieties were grouped with the control creole varieties. Some showed a small genetic distance from the tolerant, indicating genetic similarity. However, significant differences were observed among creole varieties in the greenhouse experiment. In conclusion, nine landraces can be considered tolerant to water stress according to the results of the experiments.
Keywords: ISSR markers; genetic characterization; DNA.

Tolerância ao estresse hídrico em diferentes variedades de feijão-caupi em fase de plântula

RESUMO: O feijão-caupi tem grande importância socioeconômica para a região Nordeste do Brasil. No entanto, esta região sofre com a escassez de água, levando à redução da produção. Portanto, é importante identificar variedades adaptadas às condições de estresse hídrico. Objetivou-se caracterizar variedades crioulas de feijão-caupi na fase de muda, utilizando marcadores ISSR e o método Screening Box. Utilizando marcadores ISSR, foram avaliadas amostras de DNA extraídas de 50 variedades. Dentre elas, duas foram utilizadas como testemunhas, uma tolerante (Pingo de Puro 1,2) e outra suscetível (Santo Inácio) à seca. Para identificar a variabilidade genética para tolerância à seca, foram utilizadas análises multivariadas e de agrupamento. Em seguida, foi realizado um experimento utilizando o método Screening Box, onde foi induzido déficit hídrico em 22 variedades, incluindo as testemunhas. Essas plantas foram selecionadas com base na caracterização genética realizada com o ISSR. No primeiro experimento, 45 variedades foram agrupadas com as variedades crioulas de controle. Alguns deles apresentaram uma pequena distância genética dos tolerantes, o que indica similaridade genética. No entanto, no experimento em casa de vegetação, foram observadas diferenças significativas entre as variedades crioulas. Concluindo, nove variedades crioulas podem ser consideradas tolerantes ao estresse hídrico de acordo com os resultados dos experimentos.
Palavras-chave: marcadores ISSR; caracterização genética; DNA.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] is also known as blackeyed pea and field pea. In Brazil, is named as feijão-macassar and feijão-de-corda, in the Northeast region; feijão-de-praia, feijão-da-colônia and feijão-de-estrada, in the North; and feijão-miúdo in the South (FREIRE FILHO et al., 1983). Despite its different denominations, this crop has great economic and social importance in all producing regions, both as food, and for generating employment and income for farmers employment (FROTA et al., 2008; SINGH, 2007).

The species is exploited differently, mainly as dry and green grains for human consumption (RIBEIRO et al., 2002). Also, the crop has adapted to poor soils and can be associated with soil bacteria to perform biological nitrogen fixation (TIECHER, 2016).

In 2018/19, Brazil harvested about 651500 t tons of grain, 417900 from the Northeast, the only region that showed production expansion (CONAB, 2019). Ceará, among the other states producing cowpeas in Brazil, has the largest planting area (404.200 t ha) (CONAB, 2019). For

these reasons, cowpea is an essential crop for the leading producers of the states in the North and Northeast.

However, the Northeast is located in the semiarid region, which is characterized by high temperatures (above 20 ºC on average annually) and scarce and poorly distributed rains throughout the year (from 200 to 800 mm) (JAPIASSÚ et al., 2016). According to Nascimento et al. (2011), cowpea is moderately tolerant to water deficit and waterlogging. However, drought may significantly reduce the plant production components, mainly when the water stress occurs during the flowering and fruiting stages (SOUSA et al., 2009).

Therefore, it is crucial to identify cowpea varieties tolerant to water stress since most farmers in the Northeast region cultivate under rainfed conditions, relying on rainfall for water. Thus, developing and recommending varieties tolerant to water stress may guarantee production in rainfed systems in northeastern Brazil.

Plant breeding can be initiated by evaluating and characterizing the available germplasm to know its genetic variability. It can be done through phenotyping and molecular markers, which directly reflect the genetic polymorphisms at the DNA level (SOUZA et al., 2015). The Screening Box method for phenotyping developed by Singh et al. (1999) is a quick, efficient, and simple alternative to assess many varieties for tolerance or sensitivity to water stress. In turn, among the molecular markers, the ISSR (Inter et al.), which amplifies DNA regions between identical microsatellites oriented in opposite directions (Omondi et al., 2016) through PCR (Polymerase Chain Reaction), is an excellent method of molecular markers because it is informative and highly polymorphic.

Although the Screening Box method is a quick alternative for evaluating many varieties, the researcher often faces difficulties, making the work unfeasible. Thus, using different methods, such as molecular analysis and phenotyping, may result in faster responses regarding identifying water stress-tolerant varieties. Thus, this work aimed to characterize cowpea creole varieties at the seedling stage using ISSR markers and the Screening Box method to identify genotypes tolerant to water stress.

2. MATERIALS AND METHODS

2.1. Molecular characterization of cowpea creole varieties

2.1.1 Plant material

Fifty cowpea creole varieties from different regions in Brazil were evaluated. Among them, 46 were collected in 19 municipalities from Ceará state; two were obtained from the germplasm bank at the Federal University of Ceará (UFC), Fortaleza city, Ceará; one from Sousa city, Paraíba; and one from Embrapa Meio Norte, Teresina city, Piauí (Table 1). Also, one variety was considered tolerant (Pingo de Ouro 1,2) and another susceptible (Santo Inácio) to drought, according to previous studies (RIVAS et al., 2016; LIMA et al., 2018). Both were used as controls.

2.1.2. DNA extraction

Seeds from the creole varieties presented in Table 1 were sown in polystyrene trays under a greenhouse at UFC. Seedlings with at least two expanded leaves were removed from the trays and transported to the laboratory for DNA extraction.

Analyzes were performed at UFC at the Laboratório de Biologia Molecular Aplicada à Agricultura (BIOAGRI). DNA was extracted using Doyle and Doyle's CTAB DNA extraction protocol (1990).

2.1.3. PCR and Electrophoresis

Twenty-five ISSR primers (Integrated DNA Technologies®) were used to evaluate the polymorphism of the studied genotypes. Amplification reactions were carried out with a final

volume of 15 μL, using PCR Buffer (1x), dNTPs (0.2 mM each), $MgCl_2$ (2mM), primer (0.8 μM), genomic DNA (30 ng/μL) and Taq DNA polymerase (1U) (GoTaq et al.). The THERM-1000 thermocycler (Axygen®) program consisted of an initial denaturation at 94°C for 5 min, 40 denaturation, annealing, and extension cycles, and a final extension at 72°C for 10 min. Each cycle consisted of 94°C for 1 min, 45°C, 48°C, 50°C or 55°C for 30 s (according to the primers used) and 72°C for 1 min.

The amplified products were subjected to 1.2% agarose gel electrophoresis in 0.5x TBE buffer (45 mM Tris-borate, pH 8.0, and 1 mM EDTA) at a voltage of 90 volts for 1.5 h. The gels were stained with ethidium bromide (10 ng/mL) and then visualized and photographed under UV light with a Gel Logic 212 Pro photo-imager (Carestream®)

2.1.4. Statistical analysis

Binary spreadsheets were built for each gel. The presence of bands was indicated by one, and absence by zero. This binary matrix was then analyzed in GENES software (Cruz, 2008), and a genetic dissimilarity matrix was calculated using the Jaccard similarity index (Jij):

$$J_{ij} = 1 - \frac{a}{a+b+c} \quad (01)$$

where: *a* is the presence of bands in individuals *i* and *j*, *b* is the presence of bands in individual *i* and absence in individual *j*, and *c* is the absence of bands in individual *i* and presence in individual *j*.

From the dissimilarity matrix, a dendrogram was constructed using the UPGMA (Unweighted et al. Method with Arithmetic Mean) grouping method using R software version 3.4.0 (R CORE TEAM, 2017). Also, the cophenetic correlation coefficient (CCC) was calculated to verify the fit between the dissimilarity matrix and the dendrogram, and the cut was based on Mojena (1977).

$$q_k = m + ks \quad (02)$$

where: q_k is the cut reference value, m is the mean, *is a constant (1.25),* and *s* is the standard deviation.

2.2. Evaluation for drought tolerance

The phenotyping experiment was conducted under a greenhouse at the Department of Pyrotechnics, Federal University of Ceará, Fortaleza City, Ceará. From the molecular analysis, 22 creole varieties were selected (Table 2) for to experiment. Based on the dissimilarity matrix, the creole varieties closest and most genetically distant from the control (Pingo de Ouro 1,2, the water stress-tolerant) were selected.

Table 1. Identification of cowpea landraces used in the experiment.
Tabela 1. Identificação das variedades crioulas de feijão-caupi utilizadas no experimento.

Number	Identification	Common name	Origin
Landraces used			
1	CCE-002	Chumbinho	Barbalha, CE
2	CCE-003	Maranhão	Barbalha, CE
3	CCE-004	Feijão preto do willer	Desconhecida
4	CCE-005	Desconhecido	Deputado Irapuan Pinheiro, CE
5	CCE-006	Canapu	Deputado Irapuan Pinheiro, CE
6	CCE-007	Pingo de ouro	Deputado Irapuan Pinheiro, CE
7	CCE-008	Feijão de arrancada (M) Rac	Deputado Irapuan Pinheiro, CE
8	CCE-010	Sempre verde	Deputado Irapuan Pinheiro, CE
9	CCE-012	Feijão de moita - vermelho	Guaraciaba do Norte, CE
10	CCE-013	Sempre verde	Guaraciaba do Norte, CE
11	CCE-014	Feijão moitinha Rac	Guaraciaba do Norte, CE
12	CCE-015	Feijão de corda	Guaraciaba do Norte, CE
13	CCE-018	Pitiuba	Morada nova, CE
14	CCE-019	Pingo de ouro	Morada nova, CE
15	CCE-020	Epace 10	Morada nova, CE
16	CCE-024	Feijão da Bahia Rac	Parambu, CE
17	CCE-026	Cojó	Parambu, CE
18	CCE-027	Santo Inácio	Parambu, CE
19	CCE-030	Zé Artur	Paramoti, Monte pedal, CE
20	CCE-031	Roxim miúdo Rac	Paramoti, Monte pedal, CE
21	CCE-036	Cara preta Rac	São Benedito, CE
22	CCE-037	Xique-xique	São Benedito, CE
23	CCE-038	Feijão manteiga	-
24	CCE-048	Engana mulher	Farias Brito (Cariri), CE
25	CCE-049	Feijão de corda	Farias Brito (Cariri), CE
26	CCE-051	Paulistinha	Umirim, CE
27	CCE-052	Azulão	General Sampaio, CE
28	CCE-053	Meio tardão	General Sampaio, CE
29	CCE-054	Costela de vaca	Sousa, PB
30	CCE-056	Ligeiro	General Sampaio, CE
31	CCE-059	Feijão olho de coruja	Farias Brito, CE
32	CCE-061	Feijão Canapu	Várzea Alegre, CE
33	CCE-062	Feijão sempre verde	Farias Brito, CE
34	CCE-063	Canapu - ligeiro	Farias Brito, CE
35	CCE-071	Feijão azulão	Farias Brito, CE
36	CCE-072	Manteiga	Farias Brito, CE
37	CCE-083	Feijão de corda	Trairi, CE
38	CCE-084	Vinagre	Apuiarés, CE
39	CCE-096	Russiano	Ocara, CE
40	CCE-102	Bajem mole	Baixo Acaraú, CE
41	CCE-106	40 dias	Farias Brito, CE
42	CCE-107	Galajão	Farias Brito, CE
43	CCE-109	Mané mestre	Tururu, CE
44	CCE-110	Roxão	Apuiarés, CE
45	CCE-111	Feijão Raul/ Assentamento	Madalena, CE
46	CCE-119	Concebida	Juazeiro do Norte, CE
47	CCE-120	Cabeça-de-galo	Juazeiro do Norte, CE
48	-	Pingo de Ouro 1,2	Embrapa Meio Norte/ Teresina,
49	CE-939	Paulistinha	BAGCaupi/ CCA/ UFC
50	CE-031	Pitiuba	BAGCaupi/ CCA/ UFC
Landraces were used as a control in experiments with water stress.			
48	-	Pingo de Ouro 1,2	Water stress tolerant
18	CCE-027	Santo Inácio	Water stress susceptible

Table 2. Identification of cowpea landraces used in the experiment under the greenhouse.
Tabela 2. Identificação das variedades crioulas de feijão-caupi utilizadas no experimento em casa de vegetação.

Number	Identification	Common name
Landraces		
1	CCE-003	Maranhão
2	CCE-004	Feijão preto do willer
3	CCE-005	Desconhecido
4	CCE-007	Pingo de ouro
5	CCE-008	Feijão de arrancada
6	CCE-010	Sempre verde
7	CCE-012	Feijão de moita - vermelho
8	CCE-014	Feijão moitinha Rac
9	CCE-019	Pingo de ouro
10	CCE-048	Engana mulher
11	CCE-049	Feijão de corda
12	CCE-052	Azulão
13	CCE-071	Feijão azulão
14	CCE-096	Russiano
15	CCE-102	Bajem mole
16	CCE-106	40 dias
17	CCE-110	Roxão
18	CCE-111	Feijão Raul/ Assentamento
19	CE-031	Pitiuba
20	-	Pingo de Ouro 1,2
21	CCE-027	Santo Inácio
22	CE-315	Tvu 2331
Landraces used as control		
20	-	Pingo de Ouro 1,2
21	CCE-027	Santo Inácio

Seeds from the selected creole varieties were treated with 0.5% sodium hypochlorite solution (NaClO) for one minute. They were subsequently distributed on two filter papers moistened with 2.5-fold weight before hydration and covered with a third sheet of paper. Finally, the papers containing the materials were placed in a B.O.D (Biochemical oxygen demand) (25 °C and 12 hours photoperiod). The seeds were pre-germinated on filter paper to ensure excellent uniformity during the experiment.

After four days, when most seeds emitted the radicle, the materials were transplanted to polyethylene boxes, selecting those with uniform radicle size. The boxes were 53 cm long, 37 cm wide, and 24 cm high, and they were filled with a substrate composed of sand, vermicompost, and vermiculite (at a 6:3:1 ratio by volume). The materials used in the substrate were previously sterilized to avoid the action of external agents in the experiment. In each box, eight varieties were sown with six plants, and each variety was repeated four times in the experiment.

2.2.1. Conducting the experiment and experimental design

The boxes were irrigated with 3L water to saturate the substrate, and after transplanting, the seeds were irrigated daily with 700 ml until the partial emergence of the first trifoliate leaf, which occurred seven days later. The irrigation was suspended in the experimental blocks and maintained in the control box. Then, each plant was evaluated daily for the presence and intensity of wilting symptoms, culminating in the severe wilting of all plants. Subsequently, when all plants of the susceptible creole varieties in each block seemed to be dead, irrigation was resumed, and the percentage of plants recovered was recorded.

The experiment was a randomized block design (DBC) with 22 creole varieties as treatments and four replicates. This experimental design was used to reduce errors from differences among the boxes used in the experiment and other factors that cannot be controlled, such as differences in environmental conditions and the substrate used.

The experimental plots consisted of six plants arranged in rows inside the box, and the block consisted of three boxes. Also, one box was irrigated continuously throughout the experiment and was used as a control. Inside this control box, two replicates of each landrace were used to evaluate their development without water stress and to monitor diseases and other problems that could mask the results in the experimental boxes.

Data were verified for normality and then submitted to analysis of variance, followed by the Scott-Knott test at 5% probability, to identify the susceptible and tolerant varieties to water stress.

3. RESULTS

3.1. Genetic characterization

From the 25 ISSR primers used, 16 were amplified. Among them, 14 were polymorphic bands, and two were monomorphic. Then, from the amplification of the polymorphic primers, 80 bands were obtained, and 73.75% of them were polymorphic (Table 3).

By cluster analysis, using the results obtained with the ISSR markers, groups were formed and divided based on the Jaccard coefficient. Figure 1 shows the genetic distances used to construct the dendrogram by the UPGMA method. Six groups were formed, as represented in Table 4. Group 1 contained 45 genotypes, those with higher genetic similarity to each other. The other groups were formed by only one (Groups 2, 3, 4, 5, and 6), and these creole varieties are the most distant genetically from the other, especially the CCE-110, the most divergent. The cophenetic correlation coefficient (CCC) was 0.66.

Jaccard's coefficient between the pairs of creole varieties varied from 0.071 to 0.29 (0.1639 on average), which indicates remarkable excellent genetic similarity among the studied cowpea varieties concerning the genomic region amplified by the ISSR markers. The most significant most remarkable dissimilarity was found between CCE-026 and CCE-110 (0.29), CCE-061 and CE-031 (0.27), CCE-071 and CCE-110 (0.26), and CCE-015 and CCE-110 (0.26), while the most significant similarity was between CCE-002 and CCE-005 (0.071), CCE-031 and CCE-054 (0.071), CCE-014 and CCE-111 (0.080), and CCE-012 and CCE-027 (0.085).

3.2. Evaluation for drought tolerance

When compared by the Scott-Knott test, at the level of 5% probability, the treatment means showed a significant difference in drought tolerance. As shown in Table 5, CV was 8.70%, and F values for block and treatments were significant at 1% probability.

The percentage of recovery is shown in Table 6. Some materials recovered after the resumption of irrigation. Pingo de Ouro 1,2, CCE-010, CCE-052, CCE-019, CCE-012, CCE-007, CCE-049, and CCE-008 showed severe wilting but recovered the turgor after the irrigation was resumed.

By the Scott-Knott test (Figure 2), the ten creole varieties marked in "a" were considered tolerant to water stress, while those 12 creole varieties marked in "b" were considered susceptible.

Table 3. Identification and sequence of the polymorphic ISSR primers used, number of amplified bands (A), number of polymorphic bands (P), and percentage of polymorphism.
Tabela 3. Identificação e sequência dos primers polimórficos ISSR utilizados, número de bandas amplificadas (A), número de bandas polimórficas (P) e porcentagem de polimorfismo.

Primer		Sequence (5'-3')	Number of bands		Polymorphism
			A	P	(%)
1	I-807	AGAGAGAGAGAGAGAGT	7	6	7.5
2	I-808	AGAGAGAGAGAGAGAGC	4	1	1.25
3	I-810	GAGAGAGAGAGAGAGAT	6	4	5
4	I-825	ACACACACACACACACAT	10	9	11.25
5	I-841	GAGAGAGAGAGAGAGAYC	4	4	5
6	I-842	GAGAGAGAGAGAGAGAYG	4	4	5
7	UBC-807	AGAGAGAGAGAGAGAGT	4	4	5
8	UBC-808	AGAGAGAGAGAGAGAGC	3	3	3.75
9	UBC-809	AGAGAGAGAGAGAGAGG	4	2	2.5
10	UBC-811	GAGAGAGAGAGAGAGAYC	6	3	3.75
11	UBC-825	ACACACACACACACACT	8	4	5
12	UBC-828	TGTGTGTGTGTGTGTGA	8	8	10
13	UBC-862	AGCAGCAGCAGCAGCAGC	6	3	3.75
14	UBC-873	GACAGACAGACAGACA	6	4	5
Total			80	59	73.75

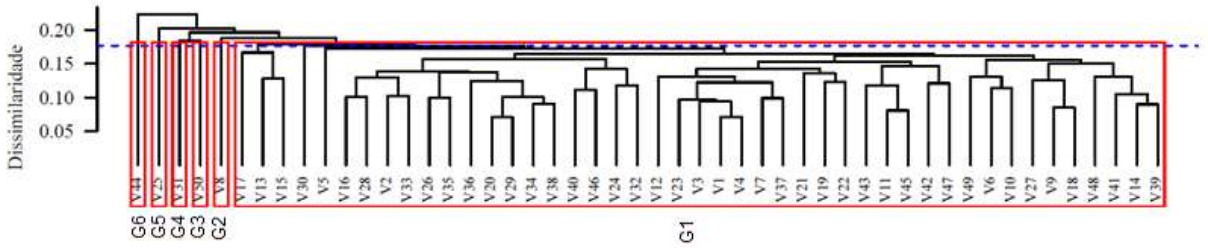

Figure 1. Dendrogram from the genetic dissimilarity matrix among the 50 cowpea landraces, using the Jaccard similarity index and the UPGMA clustering method.
Figura 1. Dendrograma da matriz de dissimilaridade genética entre as 50 variedades crioulas de feijão-caupi, utilizando o índice de similaridade de Jaccard e o método de agrupamento UPGMA.

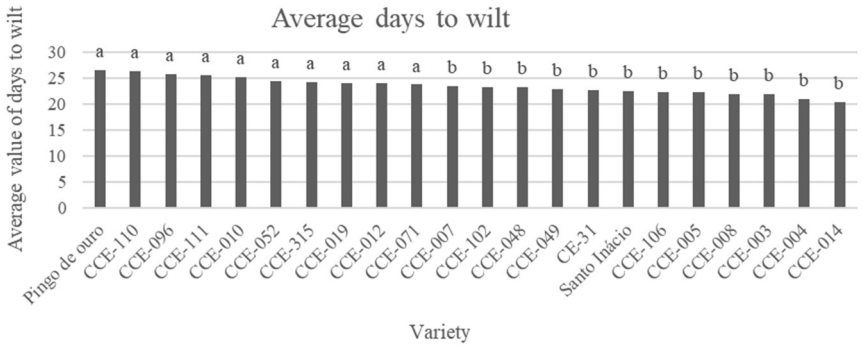

Figure 2. Graphical representation of the Scott-Knott test. Means followed by the same letter are not significantly different.
Figura 2. Representação gráfica do teste de Scott-Knott. As médias seguidas pela mesma letra não são significativamente diferentes.

Table 5. Analysis of variance for the variable analyzed in the greenhouse experiment.
Tabela 5. Análise de variância para a variável analisada no experimento em casa de vegetação.

SV	DF	SS	MQ	F
Block	3	365.105151	121.7017	28.883**
Varieties	21	235.615656	11.21979	2.6627**
Error	63	265.460404	4.213657	
Total	87	866.181212		
Mean - 23.589394		CV - 8.701875		

**significant at 1% probability.

Table 6. Percentage of plant recovery after resuming the irrigation.
Tabela 6. Percentual de recuperação das plantas após a retomada da irrigação.

Landrace	%plant recovery	Landrace	% plant recovery
Pingo de ouro	17.39	CCE-102	0.00
CCE-110	0.00	CCE-048	0.00
CCE-096	0.00	CCE-049	13.04
CCE-111	0.00	CE-31	0.00
CCE-010	17.39	Santo Inácio	0.00
CCE-052	9.52	CCE-106	0.00
CCE-315	0.00	CCE-005	0.00
CCE-019	19.05	CCE-008	4.76
CCE-012	13.04	CCE-003	0.00
CCE-071	0.00	CCE-004	0.00
CCE-007	4.35	CCE-014	0.00

4. DISCUSSION

4.1. Genetic characterization

When studying cowpea genotypes using ISSR markers, Santos et al. (2013) found a polymorphism rate of 79.41%, a high value. Dias et al. (2015) found 76% polymorphism when studying cowpea genotypes from Brazil and Nigeria using ISSR markers. Likewise, Araújo et al. (2019) characterized 57 cowpea varieties and found 76.25% polymorphism. Thus, the polymorphism value found in this study can also be considered high since it is close to the authors' values. Ghalmi et al. (2010) reported a lower polymorphism rate (62.5%) in African varieties using ISSR markers, which is also considered high. Therefore, such results indicate that ISSR markers help identify genetic variability in cowpeas.

The CCC represents the reliability of the clusters, estimating the fit between the dendrogram and the distance matrix. Rohlf (1970) established a limit of 0.70 for the grouping method to be considered adequate. The CCC of the present study was close to this limit. Araújo et al. (2019) reported the same CCC (0.66) when they analyzed creole varieties of cowpea from the state of Ceará. In turn, Amorim et al. (2008) found 0.61 when grouping 38 banana diploids. Such coefficients are obtained when many varieties are analyzed, making it challenging to represent the dissimilarity matrix in a dendrogram. On the other hand, Vaz Patto et al. (2004), in contrast to Rohlf (1970), admitted that CCC above 0.56 is adequate.

Regarding genetic distances using ISSR markers, Ghalmi et al. (2010) found a variation from 0.025 to 0.325 between the Creole varieties of cowpea in Sudan, while Dias et al. (2015) found distances ranging from 0.08 to 0.57 using RAPD (Random et al.) markers. In addition, Ali et al. (2015) evaluated 252 accessions of cowpeas from Sudan using codominant markers and observed distances ranging from 0.031 to 0.303. These results are similar to the present study and indicate a narrowing of the genetic basis of cowpeas, as previously reported by Pasquet (2000).

The low polymorphism and small genetic distances in cowpeas suggest that the domestication process caused this narrowing in the genetic base. According to Pasquet (1999), the domestication process occurred only once in cowpeas, unlike common beans (Phaseolus et al.), which suffered two major domestication processes. Also, small genetic distances were expressed even in creole varieties from different states, such as CCE-54 from Paraíba, in Pingo de Ouro 1,2 that was used as a control for water stress tolerance, and in creole varieties with visible morphological differences, like seed color and pod type. Asare et al. (2010) corroborates this statement and attribute this condition to the self-pollination mechanism in the species, which contributes to reducing its genetic variability.

Although genetic distances between the varieties were small, the ISSR markers successfully showed the genetic variability within the species. It is interesting to note that some varieties with the same name were arranged into different groups. For instance, CCE-013 and CCE-062 were arranged in Group 1, while CCE-010 was in Group 2, but all are named "Sempre Verde" and were collected in different municipalities. It may occur because of crop management, genetic variances due to differences in the microclimate, and possible crosses between the creole varieties, which can increase the genetic variability, which could be identified in the analysis.

Likewise, CCE-038 and CCE-072, as well as CCE-051 and CCE-939, were shown to be genetically distant despite having the same name, which arranged them at different branches in the dendrogram. In contrast, the Pingo-de-Ouro varieties (CCE-007, CCE-019, no code) were closer, indicating they are genetically similar. Moreover, according to the dissimilarity matrix, some varieties within Group 1 were closer to Pingo de Ouro 1,2 (Control). For instance, CCE-019 is called Pingo de Ouro and was the closest, with a 0.11765 dissimilarity index, followed by CCE-014, CCE-111and CCE-096 with 0.11881, 0.11881, and 0.12871, respectively. Other creole varieties were also genetically close to control. Thus, this criterion was used to select the cowpea creole varieties for the phenotyping test.

Therefore, 22 Creole varieties were selected based on the dissimilarity matrix: CCE-003, CCE-004, CCE-005, CCE-007, CCE-008, CCE-010, CCE-012, CCE-014, CCE-019, CCE-048, CCE-049, CCE-052, CCE-071, CCE-096, CCE-102, CCE-106, CCE-110, CCE-111, CE-031, CCE-027, CE-315 and Pingo de Ouro 1,2 (control). As a result, they were the most recommended for the greenhouse experiment to identify varieties tolerant to water stress, as they have a genetic base more similar to the control, which is a variety with tolerance already identified (Pingo de Ouro 1.2), thus increasing the probability of having genes to develop tolerance to water stress.

4.2. Evaluation for drought tolerance

According to the genetic characterization, the creole varieties CCE-019, CCE-111, CCE-96, and CCE-012 included in the tolerant group (a) also showed small genetic

distances from Pingo de Ouro 1,2. However, others like CCE-014, CCE-005, CCE-004, and CCE-008 also had small genetic distances but were grouped with susceptible creole varieties. It may have occurred because the ISSR markers randomly bind to many sites in DNA. Thus, regarding the amplified sites, the small genetic distance between the creole varieties and the tolerant ones indicates that these creole varieties are also tolerant. Only using molecular markers that are proven to be associated with water stress tolerance genes can indicate that these Creole varieties have such genes. However, obtaining these markers takes time because it needs genetic mapping and validation in different populations, besides being of higher development cost.

Singh et al. (1999) observed significant differences among 12 cowpea creole varieties from Nigeria using the same methodology adopted in this work. The authors found, by a Screening box experiment, that varietal differences in plant responses to water stress can be evaluated at the seedling stage. In addition, they observed that the results obtained with the Screening Box method correspond to those obtained in a field experiment. It suggests that the phenomenon that causes water stress at the seedling stage also manifests in cowpea's reproductive stage. Therefore, the analyses performed in this experiment can be used to indicate creole varieties with relative confidence in field experiments.

Despite this, according to Nascimento et al. (2011), just one physiological variable is insufficient to indicate drought tolerance because it is a quantitative trait expressed through different physiological responses in plants. For this reason, tests under greenhouse and field conditions should be performed later to confirm the results.

Among the varieties recovered, CCE-019 stood out with the highest recovery percentage. Thus, all plants with recovery capacity have a certain tolerance to water stress and can resume growth when rehydrated after prolonged periods of drought. These plants are the most suitable for field experiments to confirm tolerance to water stress, as they have a genetic basis more similar to the control variety, increasing the probability of having water stress tolerance genes.

In conclusion, the results showed that the prior genetic characterization to separate the materials used in phenotyping is promising since the Creole varieties varied significantly in their tolerance to water stress.

5. CONCLUSIONS

Creole varieties CCE-110, CCE-096, CCE 111, CCE 010, CCE-052, CCE-315, CCE-019, CCE-012, and CCE-071 showed tolerance to water stress in the controlled environment experiment, and they can be used in breeding programs to obtain superior varieties tolerant to drought.

Pingo de Ouro 1,2, CCE-010, CCE-052, CCE-012, CCE-007, CCE-049, and CCE-008 recovered after the resumption of irrigation.

By comparing the data of the two stages in the Screening Box, CCE-010, CCE-052, CCE-019, and CCE-012 are the most recommended regarding tolerance to water stress.

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