Special Supplement: Pulses

Non-destructive method for extracting DNA from cowpea seeds[1]

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ABSTRACT

Studies employing molecular markers require DNA extraction. For cowpea, DNA extraction is typically performed on young leaf tissue; however, this procedure requires significant time, space and resources. This study aimed to establish a protocol for extracting DNA from scarified cowpea seeds while preserving the embryo viability. Three DNA extraction protocols were evaluated to determine the most suitable non-destructive method: the CTAB method, the NaOH-Tris method and a commercial kit. The commercial kit produced DNA of higher purity, when compared to the other protocols. Nevertheless, the amplified products from the commercial kit and the CTAB protocol were highly similar. Five genetic similarity groups were identified. No significant differences were observed for the germination rate or the emergence speed of scarified seeds. The genetic variability assessment validated the efficiency of the proposed methodology. This protocol effectively preserved the embryo viability, enabling the subsequent use of the seeds in genetic breeding programs.

KEYWORDS: *Vigna unguiculata*, DNA extraction protocol, molecular markers.

RESUMO

Metodologia não destrutiva de extração de DNA a partir de sementes de feijão-caupi

Estudos com marcadores moleculares requerem a extração de DNA. No caso do feijão-caupi, essa extração é realizada a partir das folhas de indivíduos jovens. Entretanto, esse método requer tempo, espaço e recurso. Objetivou-se estabelecer um protocolo de extração de DNA a partir da escarificação de sementes de feijão-caupi, de modo a preservar a viabilidade do embrião. Para a definição da metodologia não destrutiva foram testados três protocolos de extração de DNA: método CTAB, método NaOH-Tris e um kit comercial. O kit comercial apresentou DNA de maior pureza em relação aos demais protocolos; entretanto, o produto amplificado oriundo do mesmo e do protocolo CTAB mostraram-se bastante semelhantes. Foram obtidos cinco grupos de similaridade genética. A germinação e a velocidade de emergência das sementes escarificadas não divergiu. A avaliação da variabilidade genética validou a eficiência da metodologia proposta. Esse protocolo mostrou-se eficaz ao preservar a viabilidade dos embriões, viabilizando o uso posterior das sementes em programas de melhoramento genético.

PALAVRAS-CHAVE: *Vigna unguiculata*, protocolo de extração de DNA, marcadores moleculares.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is an annual herbaceous species well adapted to a wide range of soil types, including those of low fertility (Mekonnem et al. 2022). Additionally, it demonstrates considerable tolerance to adverse conditions such as drought (Mekonnem et al. 2022). Due to this resilience, cowpea is regarded as a crucial legume for food security in tropical and subtropical regions worldwide (Mekonnem et al. 2022). Besides its substantial role as a food source, cowpea holds a significant economic importance. Brazil produced approximately 3.4 million tons of bean in the 2024/2025 harvest, with cowpea representing 612,000 tons of this total (Conab 2023).

For some time, cowpea was considered an orphan crop, due to limited research dedicated to its development. However, interest in conserving cowpea germplasm and developing genomic tools

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for breeding and improving varieties has recently increased (Boukar et al. 2019).

There is an ongoing demand for suitable, straightforward and cost-effective methodologies for preparing genomic deoxyribonucleic acid (DNA) for polymerase chain reaction (PCR) amplification from minimal quantities of plant tissue. Traditionally, DNA extraction in cowpea involves leaf samples, a process requiring seed sowing, germination, adequate plant growth and subsequent collection and processing of leaf tissues. Alternatively, recent studies have successfully isolated DNA and performed genotyping evaluations directly from seeds (Mills et al. 2020).

DNA extraction from seeds has several advantages over traditional extraction from other tissues, such as saving space in the field and reducing the effort associated with leaf sampling (Duarte et al. 2020). Additionally, genetic analyses can be expedited by pre-selecting seeds with desirable genotypes before accessions are introduced into field conditions (Ma et al. 2019). Nevertheless, it is essential that seed DNA extraction techniques do not compromise embryo viability and that the extracted DNA maintains sufficient quantity and quality for subsequent genotypic analyses (Meru et al. 2013).

With DNA of adequate quality and quantity, the genetic variability among cowpea accessions can be assessed effectively using molecular markers (Joshi et al. 2022). PCR-based molecular markers such as single nucleotide polymorphism (SNP), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) have been widely used (Paiva et al. 2019, Dwiningsih et al. 2020). ISSR markers are particularly advantageous, as they do not require prior sequence knowledge and have been effectively applied in plant systematic studies, especially for assessing genetic diversity in germplasm collections (Shaygan et al. 2021).

Therefore, this study aimed to develop a DNA extraction protocol from cowpea seeds that preserves embryo viability post-extraction for subsequent use in breeding programs. Additionally, the genetic variability among accessions from the cowpea germplasm bank at the Universidade Federal do Ceará was evaluated to validate the proposed methodology and inform future breeding crosses.

MATERIAL AND METHODS

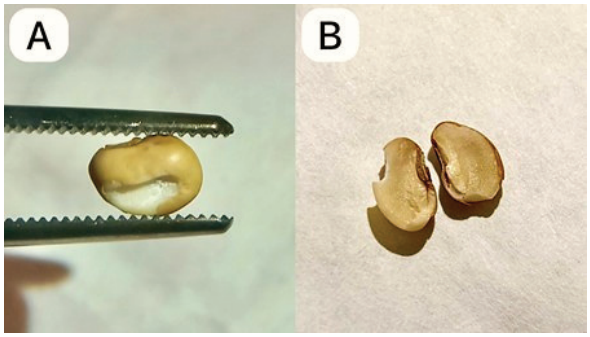
Seeds from ten cowpea accessions, obtained from December 2023 to May 2024, were used to develop a methodology for extracting DNA from seeds (Table 1). These seeds were provided by the cowpea germplasm bank at the Universidade Federal do Ceará.

For the extraction of genetic material, seeds underwent scarification (Figure 1) using a Dremel 3000 micro-grinder equipped with a 2-mm spherical diamond-tipped drill bit (nº 7105). Scarification involved drilling a hole into the lateral side of the seed, penetrating only one cotyledon previously identified as not containing the embryo (Figure 1).

After obtaining samples from the scarified seeds, two laboratory protocols and one commercial kit were tested: the sodium hydroxide-Tris (NaOH-Tris) method (Von Post et al. 2003), the cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle 1987) and the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA).

Table 1. Cowpea genotypes used in the amplification tests.

Identification	Accession ID	Common ID	Class	Subclass
1	CE-02	Bengala	Colored	Buttercream
2	CE-942	BRS Juruá	Colored	Green
3	CE-943	BRS Aracê	Colored	Green
4	CE-957	MNC-01-627D-65-1	White	Smooth white
5	CE-978	BRS Tumucumaque	White	Smooth white
6	CE-999	MNC 03-720-C-31	White	Fradinho (black-eyed)
7	CE-1002	MNC 01-627F-14-5	White	White
8	BRS Pujante	BRS Pujante	Colored	Evergreen
9	Pingo de Ouro	Pingo de Ouro	Colored	Canapu
10	Paulistinha	Paulistinha	Colored	Canapu



Photos: Victoria de Oliveira Santos

Figure 1. Seed drilled with a 2-mm drill bit. A) surface view of the scarified seed; B) cross-sectional view of the seed showing the internal embryo.

To establish the minimum amount of tissue necessary to yield high-quality DNA, extractions were conducted using different sample sizes. DNA extractions performed with the commercial kit used sample weight ranging from 0.6 to 23 mg. For the other methods, they ranged from 8 to 40 mg for the NaOH-Tris method (Von Post et al. 2003) and from 7 to 28 mg for the CTAB method (Doyle & Doyle 1987).

After each extraction, the DNA obtained was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific). Subsequently, raw DNA samples were visualized on a 0.8 % agarose gel stained with ethidium bromide, and DNA variations among accessions were identified using a 1 Kb molecular marker (Invitrogen).

To verify seed viability after scarification, emergence tests were conducted using two treatments: scarified seeds and non-scarified seeds (control) at different time intervals. The first test was carried out immediately after seed scarification. Subsequent tests occurred at 2, 4 and 6 months after scarification.

Each emergence test included 20 scarified and 20 non-scarified seeds from each accession, totaling 200 scarified and 200 control seeds per test.

The experiment was conducted in a greenhouse, where seeds from each treatment were sown in trays containing autoclaved sand substrate. Seeds were evaluated daily for 8 days to monitor the emergence speed and germination rates. Data obtained on seedling emergence were used to calculate the emergence speed (ES; days) according to Edmond & Drapala (1958): $ES = [(N1.G1) + (N2.G2) + ... (Nn.Gn)]/(G1 + G2 + ... Gn)$, where G is the number of emerged seedlings observed at each count and N the number of days from sowing to each count.

The results obtained from the four emergence tests were subjected to analysis of variance (Anova) at 5 % of significance to compare the two treatments (scarified and control seeds) and the storage intervals for scarified seeds. Significant differences identified by Anova were further analyzed using the Tukey mean comparison test at 5 % of significance. The residuals were tested using the Shapiro-Wilk test to verify data normality and the Levene's test to check for heteroscedasticity, ensuring that data met the requirements for Anova.

Germination data were analyzed using a generalized linear model (GLM) with binomial distribution to compare the two treatments (scarified and control seeds) and the storage intervals for scarified seeds. All analyses were performed using the RStudio software.

To verify the viability of the DNA extracted from seeds, genetic variability was assessed in the ten accessions used in this study (Table 1). DNA was extracted following the aforementioned scarification protocol, using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA).

Initially, DNA amplification tests were performed using 50 ISSR primers, from which primers showing polymorphism were selected. For PCR amplification, a reaction mix was prepared containing sterile deionized water, PCR Buffer (1X), dNTPs (0.2 mM each), $MgCl_2$ (2 mM), ISSR primer (0.8 µM), genomic DNA (30 ng μL^{-1}) and Taq DNA polymerase (1 U), with a total final volume of 25 µL. Amplification reactions were carried out in a Labnet MultiGene Gradient thermal cycler with the following conditions: initial denaturation at 94 ºC for 5 min; 40 cycles consisting of denaturation at 94 ºC for 1 min, annealing for 30 seconds (temperature varied according to primer) and extension at 72 ºC for 1 min; and final extension at 72 ºC for 10 min.

Amplified products were electrophoresed on 1.8 % agarose gel stained with safer dye (Kasvi), in 1X TBE buffer (45 mM Tris-borate pH 8.0 and 1 mM EDTA), at 110 V for 90 min. A 1 Kb molecular marker (Invitrogen) was used as a reference.

Following electrophoresis, gels were visualized using a Kasvi LED transilluminator (model K33-333) for subsequent analyses.

For the molecular data analysis, a binary data matrix was constructed from the molecular markers visualized on the gel, indicating the presence (1) or absence (0) of bands. Subsequently, cluster analysis

was performed using the NTSYSpc software. First, a similarity matrix was generated, and then a dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA). The cutoff point was defined as the average genetic distance from the matrix. Finally, the cophenetic correlation coefficient (CCC) was estimated to validate the clustering method. The polymorphic information content (PIC) was calculated for each primer according to Anderson et al. (1993): $PIC = 1 - \Sigma pij2$, where pij is the frequency of the allele "j" for marker "i".

RESULTS AND DISCUSSION

The results obtained with the commercial kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, WI, USA) were superior, when compared to the two tested in-house protocols. The DNA quantification indicated a satisfactory DNA quality, with absorbance ratios (A260/A280) ranging from 1.79 to 2.22, and A260/A230 ratios between 1.10 and 2.11 (Table 2).

On the gel resulting from electrophoresis of the extracted DNA samples (Figure 2), sample A5, obtained using the commercial kit, was the most intact, when compared to the other samples. This specific band corresponds to the sample with the greatest mass among those extracted using the commercial kit.

Additionally, despite distinct bands being observed in the DNA extracted by the CTAB method, significant smearing and well retention were evident, indicating the presence of impurities. Regarding the NaOH-Tris method, despite obtaining high DNA concentrations in quantification, the gel electrophoresis showed degraded, low-quality DNA.

In DNA extraction studies conducted on pumpkin seeds, DNA extracted with commercial kits (E.Z.N.A. and Favorgen) showed a superior quality, if compared to other tested methods, such as the CTAB protocol (Martinez et al. 2021). The A260/A230 absorbance ratios obtained in that study ranged between 1.76 and 1.88, values similar to those obtained in the current study.

The CTAB method (Doyle & Doyle 1987) did not yield results as satisfactory as those obtained using the commercial kit. Quantification revealed

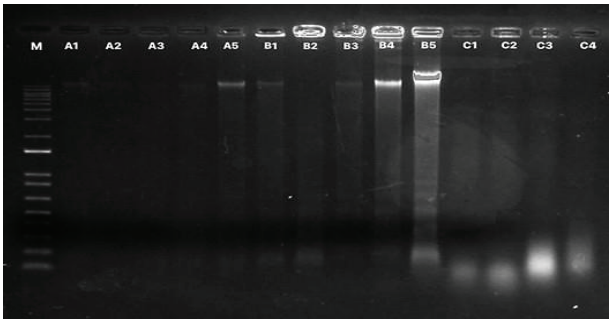


Figure 2. Raw DNA results on a 0.8 % agarose gel from extraction using the commercial kit and tested protocols. M: molecular marker 1 Kb (Invitrogen); A1-A5: Wizard® Genomic DNA Purification Kit (Promega); B1-B5: Doyle & Doyle (1987); C1-C4: Von Post et al. (2003).

Table 2. DNA extraction quantification results.

Identification	Sample weight (mg)	DNA concentration (ng ul^{-1})	Absorbance 260/280	Absorbance 260/230
A1	0.6	170.7	2.10	2.11
A2	5.3	16.2	2.22	1.82
A3	3.4	277.1	2.09	1.98
A4	7.4	392.4	2.10	2.04
A5	23.0	129.6	1.79	1.10
B1	7.0	664.0	1.64	0.63
B2	14.0	2,147.5	1.46	0.68
B3	22.0	1,785.9	1.93	1.31
B4	28.0	972.7	1.48	1.32
B5	23.0	3,583.3	1.75	0.86
C1	8.0	1,720.2	1.43	0.59
C2	14.0	2,087.3	1.56	0.46
C3	26.0	2,911.4	1.47	0.53
C4	40.0	5,015.8	1.39	0.42

The letter "A" refers to DNA extracted using the commercial kit, "B" to DNA extracted using the CTAB method and "C" to samples extracted using the NaOH-Tris method.

a DNA of moderate quality, with A260/A280 absorbance ratios ranging between 1.46 and 1.93, and A260/A230 ratios between 0.63 and 1.32.

In the study by Gao et al. (2008), who extracted DNA from maize seeds, the DNA obtained using the CTAB protocol exhibited absorbance patterns similar to those observed in this study. The A260/A280 absorbance ratios were generally close to 2.0, whereas the A260/A230 ratios ranged from 0.3 to 1.5. The A260/A230 ratio is associated with contamination by organic compounds such as carbohydrates. Thus, this ratio serves as a critical quality indicator for DNA extracted from seed endosperm, which typically contains significant levels of carbohydrates.

Quantification results from DNA extracted using the NaOH-Tris method (Von Post et al. 2003) indicated unsatisfactory quality, with A260/A280 absorbance ratios ranging from 1.39 to 1.56, and A260/A230 ratios between 0.42 and 0.59. Additionally, gel electrophoresis (Figure 2) revealed negative outcomes, displaying only smeared patterns without distinct bands, indicative of significant DNA degradation.

The plant species plays a crucial role in determining the suitability of a DNA extraction protocol, as seeds from different species vary in chemical composition and structural characteristics (Santos et al. 2021). Unlike barley seeds, phytochemical analyses of grains and seed coats from various cowpea varieties have revealed high levels of phenolic compounds in these seed parts (Santos et al. 2021). Such phenolic compounds tend to oxidize and degrade DNA during extraction, significantly limiting the application of certain extraction protocols. This factor likely accounts for the observed inefficiency of the NaOH-Tris method in extracting DNA from cowpea seeds, contrasting with the satisfactory outcomes reported with barley seeds. The differential distribution of phenolic compounds among species possibly explains this variation. In barley, phenolic compounds are predominantly concentrated in the husks rather than in the seeds themselves (Martins et al. 2023).

Ma et al. (2019), using the NaOH-Tris method for DNA extraction from wheat seeds, also reported undetectable DNA on agarose gel electrophoresis. This outcome likely resulted from the DNA's low purity, as impurities can interfere with DNA binding to nucleic acid stains during electrophoresis (Ma et al. 2019).

The ISSR primers successfully amplified the DNA samples extracted using both the commercial kit and the CTAB method (Doyle & Doyle 1987). Notably, PCR products from these two methods exhibited very similar banding patterns. In contrast, no amplification occurred with DNA samples extracted using the NaOH-Tris method (Von Post et al. 2003), where only smeared patterns were visible on the gel, confirming DNA degradation during the extraction process.

DNA extracted from maize seeds by Gao et al. (2008) using the NaOH-Tris method yielded reasonable amplification results, indicating a potential applicability in molecular marker studies. However, the maize seed composition closely resembles the barley seed, the original species employed by Von Post et al. (2003) for the NaOH-Tris method. Thus, the NaOH-Tris method may be effective in genetic studies of cereals, but seems to be unsuitable for legumes such as cowpea.

Considering the successful amplification of DNA extracted using the commercial kit and the CTAB method, both protocols can be recommended as the most suitable for molecular marker analyses in cowpea. The final choice between these methods may be guided by cost-benefit analysis. Furthermore, employing seed-based extraction methodologies facilitates high-throughput processing of numerous samples daily, optimizing operational time, reducing resource consumption and eliminating the need for seedling cultivation solely for DNA extraction from leaf tissues (Castro et al. 2025).

The results obtained from the Anova analysis of the emergence test data concerning the treatment applied to seeds indicated no statistically significant differences between the emergence speed of control and scarified seeds (p-value = 0.0592). However, seed scarification tended to positively affect this variable.

Mechanical scarification exposes the endosperm, increasing its permeability to air and water, and facilitating gas exchange (Mousavi & Rezaei 2011). This increased permeability might explain the trend towards higher emergence speeds in scarified seeds, in relation to control seeds, although the difference was not statistically significant.

The interaction between scarification and storage time was not significant (p-value = 0.8344). Nevertheless, storage duration strongly influenced the emergence speed (p-value = 4.65×10^{-14}). Given

the significant p-value, data related to storage time underwent the Tukey test.

The Tukey test indicated that seeds evaluated at two months after scarification had the lowest emergence speed, when compared to other intervals (Figure 3). In contrast, seeds stored for six months exhibited the highest emergence speed, significantly differing from seeds stored for two and four months (p-values = 0.0001 and 0.00185, respectively). Furthermore, the emergence speed of seeds germinated immediately after scarification did not statistically differ from seeds germinated at four and six months after scarification (Figure 3).

Regarding germination rate data, the generalized linear model (GLM) analysis indicated high germination rates in both treatments, ranging from 96 to 98 %, with no statistical difference between treatments (p-value = 0.78). Similarly, no significant differences were found regarding storage time of scarified seeds or the interaction between storage time and treatment (p-values of 0.855 and 0.969, respectively).

Among the 50 ISSR tested primers, 25 showed polymorphism and were selected to assess genetic variability among the 10 cowpea accessions.

Amplification with the 25 polymorphic primers generated 230 bands, with 67.62 % of polymorphism. The percentage of polymorphic bands ranged between 14.28 and 100 % among the primers (Table 3).

When assessing genetic diversity in local cowpea varieties in India using ISSR markers, Saxena & Tomar (2020) reported 88.93 % of polymorphism, whereas Joshi et al. (2022) reported 89.21 %. Conversely, Santos et al. (2020), evaluating cowpea genetic diversity in Mexico, observed an average polymorphism of 67.7 %, a value very similar to that found in the present study. In Brazil, Araújo et al. (2019) found 76 % of polymorphism among 52 cowpea populations.

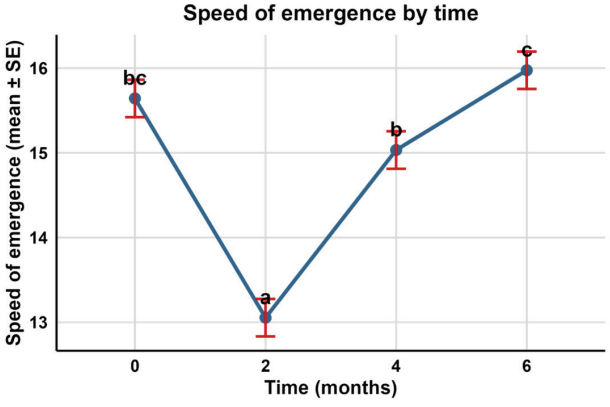


Figure 3. Emergence speed as a function of scarified seed storage duration. Points with the same letter indicate no significant differences according to the Tukey test at 5 % of probability.

The differences in polymorphism across these studies may be explained by the origin and size of the analyzed cowpea populations, as well as the specific ISSR primers chosen (Santos et al. 2020). Due to the fact that cowpea cultivation occurs in diverse geographic regions worldwide, local environmental factors can influence genetic diversity, promoting distinct adaptations to adverse conditions. Additionally, diverse domestication histories and the effects of natural or artificial selection could account for these polymorphic differences observed among populations from different locations.

In this study, the average polymorphic information content (PIC) was 0.72, with primer-specific values ranging from 0.50 to 0.93 (Table 3). According to Botstein et al. (1980), PIC values greater than 0.5 are considered highly informative; thus, the primers used in this study can be classified as highly informative.

Based on the obtained polymorphic bands, a dendrogram was constructed using the UPGMA method from the similarity matrix, employing a cutoff point of 0.66 calculated from the average of the genetic distance matrix. Four genetic similarity groups were identified (Figure 4).

The most similar genotypes were the accessions 4 (CE-957) and 6 (CE-999), as well as 4 (CE-957) and 7 (CE-1002), exhibiting similarities of 0.75 and 0.74, respectively. Conversely, the most genetically divergent accessions were 3 (BRS Aracê) and 10 (Paulistinha), showing a genetic distance of 0.36 (Table 4).

The validation of the dendrogram by calculating the cophenetic correlation coefficient (CCC) resulted in a value of 0.84. According to Rohlf (1970), a CCC above 0.7 indicates that the clustering method employed is suitable; hence, the UPGMA clustering method applied here is validated.

Group I included the accessions 4 (CE-957) and 7 (CE-1002), which share the same parental lines (TE99-496-1F and TE97-411-15F-2-1), indicating a close genetic lineage. Additionally, the accession 6 (CE-999) shares a related breeding lineage (MNC-

Table 3. Primer sequences, total bands generated and number of polymorphic bands from ISSR primers used to characterize cowpea genotypes.

Primers	Full sequence (5'-3')	Ta (ºC)	TB	PB	P (%)	PIC
I807	$(AG)_8$FT	43.0	7	2	28.57	0.93
I808	$(AG)_8$C	44.8	7	4	57.14	0.66
I809	$(AG)_8$G	44.2	10	3	30.00	0.76
I815	$(CT)_8$G	42.8	6	5	83,33	0.83
I816	$(CA)_8$T	46.1	10	4	40.00	0.61
I818	$(CA)_8$G	49.0	7	7	100.0	0.75
I822	$(TC)_8$A	43.0	6	6	100.0	0.79
I825	$(AC)_8$T	47.4	9	7	77.77	0.57
I827	$(AC)_8$G	49.0	7	4	57.14	0.50
I830	$(TG)_8$G	48.7	10	2	20.00	0.83
I834	$(AG)_8$YT	45.2	15	15	100.0	0.73
I836	$(AG)_8$YA	44.9	7	4	57.14	0.85
I841	$(GA)_8$YC	44.5	11	7	63.63	0.77
I844	$(CT)_8$RC	44.6	8	8	100.0	0.69
I849	$(GT)_8$YA	47.4	11	11	100.0	0.65
I850	$(GT)_8$YC	48.7	9	7	77.70	0.56
I851	$(GT)_8$YG	49.0	7	4	57.14	0.72
I856	$(AC)_8$YA	48.8	12	12	100.0	0.57
I857	$(AC)_8$YG	50.3	17	15	88.23	0.61
I858	$(TG)_8$RT	49.1	10	8	80.00	0.71
I873	$(GACA)_4$	43.4	7	1	14.28	0.96
I878	$(GGAT)_4$	43.5	10	10	100.0	0.89
I880	$(GGAGA)_3$	43.9	10	8	80.00	0.76
I881	$(GGGTG)_3$	55.3	7	2	28.57	0.75
I887	DVD$(TC)_7$	42.4	10	5	50.00	0.73
Total			230	161	67.62	0.72

Ta: annealing temperature; Y = C or T; R = A or G; D = A, G or T; V = A, C or G; TB: total bands; PB: polymorphic bands; P: percentage of polymorphism; PIC: polymorphic information content.

01-625D-10-2) with the accession 4 (CE-957) (MNC-01-627D-65-1) (Silva 2023).

In Group II, the accession BRS Aracê (accession 3) shares a common ancestor (MC00-599F) with BRS Juruá (accession 2) (Embrapa 2009).

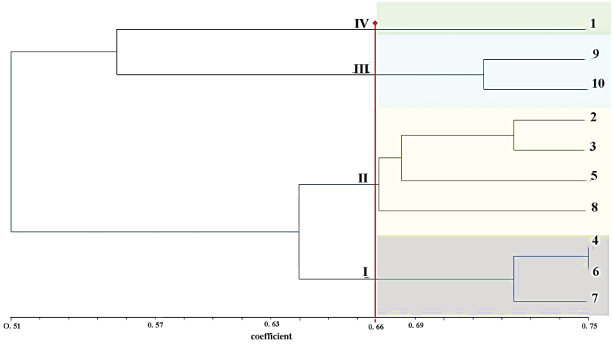


Figure 4. Genetic similarity dendrogram (UPGMA) of 10 cowpea accessions based on 25 ISSR primers. The red arrow indicates the dendrogram cutoff point (0.66). The numbers 1 to 10 are detailed in Table 1.

The accession 3 (BRS Aracê) also has a parental lineage (MNC99-537F) related to the accession 5 (BRS Tumucumaque) (Carvalho & Freire Filho 2009). Although the accession 8 (BRS Pujante) does not share common ancestors, it exhibits similar agronomic characteristics (semi-prostrate growth, crop cycle, yield and adaptation regions) to the accessions 2 (BRS Juruá) and 3 (BRS Aracê) (Santos et al. 2007).

Group III consisted of the accessions 9 (Pingo de Ouro) and 10 (Paulistinha). Both genotypes share frequent agronomic traits, such as purple flowers, lanceolate central leaflets and semi-erect plant architecture (Santos et al. 2007).

An isolated group (Group IV) was formed by the accession 1 (CE-02), which exhibited distinct characteristics, when compared to the others, such as being the only representative of the buttercream subclass with white seeds and a black hilum, combining high yield with early growth cycle. The formation of a single-individual group, as highlighted

Table 4. Genetic similarity matrix among cowpea accessions.

Accession*	1	2	3	4	5	6	7	8	9	10
1	-	-	-	-	-	-	-	-	-	-
2	0.59	-	-	-	-	-	-	-	-	-
3	0.52	0.72	-	-	-	-	-	-	-	-
4	0.48	0.59	0.59	-	-	-	-	-	-	-
5	0.56	0.65	0.68	0.64	-	-	-	-	-	-
6	0.54	0.66	0.59	0.75	0.59	-	-	-	-	-
7	0.55	0.66	0.60	0.73	0.69	0.70	-	-	-	-
8	0.60	0.68	0.63	0.59	0.67	0.67	0.65	-	-	-
9	0.60	0.51	0.43	0.51	0.55	0.52	0.55	0.57	-	-
10	0.50	0.43	0.36	0.44	0.46	0.44	0.44	0.57	0.70	-

* The numbers 1 to 10 are detailed in Table 1.

by Lima et al. (2015), indicates that such individuals possess a greater genetic divergence, when compared to others.

Although the genetic distances among accessions were small, ISSR markers effectively demonstrated a genetic variability within the studied cowpea population.

CONCLUSION

The assessment of genetic variability among cowpea accessions validated the efficiency of the proposed methodology, enabling the establishment of a protocol for DNA extraction directly from seeds. This protocol proved effective in preserving embryo viability for subsequent germination, thus allowing seeds to be used later in breeding programs.

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