Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Comparative study of the physiological and health quality of traditional and biofortified cowpea seeds

Estudo comparativo da qualidade fisiológica e sanitária de sementes tradicionais e biofortificadas de feijão-caupi

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Abstract

Cowpea is one of the main crops in family agriculture, especially in the Northeastern region of Brazil, and it is expanding to other regions in Brazil. The use of seeds with low physiological and health quality is reflected in the plant development and consequently yield, making it important to study the seed physiological and health quality. The objective of the present study was to assess the physiology and health of traditional and biofortified cowpea seeds. The traditional cowpea varieties (Angelim, Mercado and Manteguinha) and the biofortified cowpea cultivars (BRS Aracê, BRS Xique-Xique and BRS Tumucumaque) were assessed for the following physiological parameters: water content (WC), first count (FC), germination test (G), germination speed index (GSI), seedling emergence in the greenhouse (E), emergence speed index (ESI), seedling aerial part and root length (APL and RL) and electric conductivity test (EC). The seed health quality was assessed by the *Blotter Test.* The water content present in the seeds of the traditional and biofortified varieties ranged from 10% to 14%. All the traditional and biofortified varieties showed high germination and emergence value in the greenhouse. The germination and emergence speed indexes indicated the BRS Aracê and BRS Xique-Xique cultivars as the most vigorous. In the health tests the highest indexes were the storage fungi *Aspergillus* sp., *Cladosporium* sp. and *Penicillium* sp., with the highest prevalence in the BRS Tumucumaque variety, which was probably related to the higher water content present in this variety.

Keywords: *Vigna unguiculata*, germination, vigor, storage fungi.

Resumo

O feijão-caupi é uma das principais culturas da agricultura familiar, especialmente da região Nordeste, e encontra-se em expansão para as demais regiões do Brasil. O uso de sementes com baixa qualidade fisiológica e sanitária refletem no desenvolvimento das plantas e consequentemente na produtividade. Desse modo ressalta-se a importância do estudo da qualidade fisiológica e sanitária das sementes. Esse trabalho objetivou avaliar a fisiologia e sanidade de sementes tradicionais e biofortificadas de feijão-caupi. As variedades tradicionais (Angelim, Mercado e Manteguinha) e as biofortificadas (BRS Aracê, BRS Xique-Xique e BRS Tumucumaque) de feijão-caupi foram avaliadas quanto aos seguintes parâmetros fisiológicos: Teor de água (T.A), Primeira contagem (PC), Teste de germinação (G), Índice de velocidade de germinação (IVG), Emergência de plântulas em casa de vegetação (E), Índice de velocidade de emergência (IVE) Comprimento da parte aérea e raiz da mudas (CPA e CR) e Teste de Condutividade Elétrica (CE). Para avaliação da qualidade sanitária das sementes foi adotado o *Blotter Test*. O teor de água presente nas sementes das variedades tradicionais e biofortificadas, encontra-se entre 10% a 14%. Todas as variedades tradicionais e biofortificadas apresentaram alto valor de germinação e emergência em casa de vegetação. O índice de velocidade de germinação e emergência indicaram as cultivares BRS Aracê e BRS Xique-Xique as mais vigorosas. Nos testes de sanidade a maior incidência foram dos fungos de armazenamento *Aspergillus* sp., *Cladosporium* sp. e o *Penicillium* sp., com maior prevalência na variedade BRS Tumucumaque, provavelmente relacionado ao maior teor de água presente nessa variedade.

Palavras-chave: *Vigna unguiculata*, germinação, vigor, fungos de armazenamento.

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Received: August 10, 2023 – Accepted: November 20, 2023

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an expending crop in Brazil but most of the production is concentrated in the Northern and Northeastern regions. Its production in the country is estimated at around 630.8 thousand tons in the 2021/2022 growing season (CONAB, 2022). The expansion of the planted area of this crop has emphasized the importance of using quality seeds in plantings, because the success of these systems starts with the choice of adequate seeds.

The seed is one of the most important inputs whatever the agricultural production system. According to Minuzzi et al. (2010), seeds with high vigor have quicker metabolic processes, promoting faster and more uniform emission of the primary root in the germination process and high growth rate, producing seedlings with larger initial size.

Family agriculture, responsible for most of the cowpea production, often uses seeds of varieties traditionally cultivated in the region, whose genetic importance is the potential for improving other cultivars (Gomes Filho et al., 2017). In addition to the traditional and biofortified varieties, there are the biofortified cultivars, that have high iron, zinc and protein contents (Rocha et al., 2014), which are characteristics resulting from the process of nutritional biofortification in the crop that arose as one of the strategies to ensure nutrient intake, mainly micronutrients, reached the minimum (recommended) values, especially for the most needy part of the population, who, consequently, most suffer from nutritional deficiencies (Gonçalves et al., 2015).

Physiological and health quality assessment is an essential component to control adequate seed use and gives information to detect and solve problems during the productive process and also informing on the seed performance (Marcos Filho, 2015). Seed quality influences the germinative capacity, vigor and consequently yield, and seeds of high and good quality are those considered to meet these high requirements, treated in an appropriate manner, with good appearance and an adequate degree of moisture (Silva et al., 2023). The most used tests include the germination, speed index, cold test, emergence speed index and field emergence.

Associated to seed quality is the need to know the diseases that attack the cowpea, potentially transmitted by the seeds. Several fungal diseases such as grey stem rot, wilts, fusarium and sclerotic rots, for example, may cause plant death that cause damage throughout the crop cycle and reduce yield (Athayde Sobrinho, 2016). Thus assessment of seed health quality is one of the aspects that most needs attention in the productive systems and the detection of these organisms becomes one of the most important tools in health management of diseases and their dissemination in new planting areas (Barrocas and Machado, 2010).

Although some authors have reported aspects of cowpea seed health (Rodrigues and Menezes, 2002; Silva, 2006) and physiological quality (Silva, 2011; Assis et al., 2019; Silva et al., 2019; Raisse et al., 2020), there is little information on the comparative study of traditional and biofortified cowpea varieties. Thus it is essential to know the quality of the seeds used in the plantations to improve production efficiency in this crop, especially for small farmers. Therefore, objective of the present study was to compare the seed physiological and health profile of traditional and biofortified cowpea varieties.

2. Materials and Methods

The experiments were carried out in the Plant Pathology Laboratory and greenhouse at the State University of Maranhão – UEMA, Campus Paulo VI, São Luís, MA, Brazil, coordinates 2° 59"19" S, 44° 21"20" W, from March to September 2020.

The traditional seeds were obtained from cowpea producing properties in the Angelim community, in the municipality of Balsas/MA, where the Angelim, Mercado and Manteguinha genotypes were collected. The biofortified seeds were the BRS Aracê, BRS Xique-xique and BRS Tumucumaque cultivars, donated by the Brazilian Enterprise for Agricultural Research - Empresa Brasileira de Pesquisa em Agropecuária (Embrapa Meio-Norte and Embrapa Cocais).

To assess the physiological quality of traditional and biofortified cowpea seeds the following tests were used: a) Water content – expressed on wet base and determined according to the recommendations of the Seed Analysis Rules (Regras de Análise de Sementes -RAS) (Brasil, 2009a), by the chamber method at 105 ± 3 °C, for 24h. b) First seed count – the assessment was made on the fifth day after setting up the test and the result was expressed in percentage of normal seedlings. c) Germination test – carried out following the Seed Analysis Rules (Regras para Análise de Sementes) (Brasil, 2009a). Four 50-seed replications were used, distributed on two sheets of germitest paper and covered by a third sheet, forming rolls that were moistened with distilled water. The rolls were placed inside polyethylene bags to maintain moisture and taken to BOD. The seedlings were assessed on the fifth day, for the first count, and on the eighth day, final count, and the results obtained were expressed in percentage of normal seedlings. d) Germination speed index – assessed while performing the germination test, it was assessed every day, from root emission to the last count established by the RAS (Vieira and Carvalho, 1994) for germination of the cowpea crop. The formula proposed by Maguire was used for the calculation (1962).

The following were also assessed: e) seedling emergence in the greenhouse – carried out with four 50-seed replications, using autoclaved sand as substrate, in the greenhouse. For the assessment, present emerged seedlings were considered and the results expressed in percentage (Vieira and Carvalho, 1994). f) Emergence speed index – carried out together with the seedling emergence test and counted daily, starting with the first emerged seedling. The emergence speed index (IVE) was calculated according to Maguire (1962). g) Seedling aerial part and root length: the aerial part of the seedlings was measured, using a ruler graded in millimeters, from the stem insertion to the tip of the apical shoot. The root length was measured from the stem insertion to the end of the main root and the measurements were taken at the end of the germination test on 20 random seedlings.

The results were expressed in cm. h) Electrical conductivity test carried out on four 50-seed replications in 200 ml plastic cups, containing 75 ml deionized water and placed in a BOD-type germinator at 25ºC for 24h. After this period the electrical conductivity was read in the imbibing solution using a conductivity meter (Vieira and Carvalho, 1994).

The seed health quality was assessed by the *Blotter Test* method according to the Seed Health Analysis (Manual de Análise Sanitária de Sementes) (Brasil, 2009b). First, the seed samples were disinfested for five minutes by immersion in sodium hypochloride solution (NaOCl), with 1.5% active chlorine, followed by two rinses in sterilized water.

The seeds were then plated in gerbox-type plastic boxes, previously disinfested by exposing to ultraviolet light (UV) for 20 minutes, containing two layers of sterilized filter paper moistened with sterilized distilled water. The study sample was composed of 200 seeds per cultivar using 25 seeds per gerbox. The seeds were incubated under 12h photoperiod, at a temperature of around 25±2 ºC, for seven days.

The fungi were identified by the morphological characteristics observed under stereoscopic and optical microscopes. When direct identification of the fungus was not possible, the colonies that developed on the seeds were transferred to Potato-Dextrose Agar culture medium (BDA), and microcultures were prepared for their later identification according to their reproductive and vegetative structures, using the dichotomous keys: Dematiaceous Hiphomycetes (Ellis, 1971), The Fusarium Laboratory Manual (Leslie and Summerell, 2006), Illustrated genera of imperfect fungi (Barnett and Hunter, 1998).

A complete randomized design was used with six varieties and four replications. The data of the physiological analyses were submitted to analysis of variance and the means were compared by the Tukey at 5% de probability using the INFOSTAT software (Di Rienzo et al., 2018).

3. Results

The water content in the cowpea seeds in the traditional varieties ranged from 10.6% to 11.62%, and in the biofortified varieties from 11.13% to 14.93% (Table 1). The traditional and biofortified BRS Aracê and BRS Tumucumaque cultivars showed the biggest water content values among all the varieties tested.

All the traditional varieties and biofortified cultivars showed minimum germination percentage of at least 94%. The first count data demonstrated that all the varieties and cultivars already showed germination of over 80% on the fifth count day carried out in the tests (Table 1).

The BRS Aracê and BRS Xique-Xique cultivars showed the highest germination speed index, 16.57 and 16.32 respectively (Table 1).

The root length of the BRS Tumucumaque cultivar showed the lowest value (7.28 cm), while the Angelim and BRS Xique-Xique cultivars showed the highest values (13 cm). For the aerial part length the Mercado variety showed the lowest value (9.01 cm) and BRS Xique-Xique was the most developed variety (15.48 cm) (Table 1).

The emergence test values for the cowpea seeds in the traditional varieties ranged from 96% to 99%, and in the biofortified varieties the values were 86.5% to 98.5% (Table 2). All the varieties showed high emergence value in the greenhouse. BRS Tumucumaque showed significantly lower emergence than the other cultivars.

The BRS Tumucumaque cultivar showed the highest electrical conductivity (CE), among all the varieties, with 77.12 µS cm^{-1} g^{-1}, therefore showing a larger quantity of leachates.

The values found for the traditional varieties did not differ statistically and were lower than the EC of biofortified cultivars, demonstrating greater vigor by the electrical conductivity test (Table 2).

The following fungi were identified in the health test: *Aspergillus niger* Tiegh; *Cladosporium herbarum* (Pers.) Link; *Fusarium* sp.; *Penicillium* sp.; *Phoma* sp.; *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill.; *Colletotrichum* sp.; *Macrophomina phaseolina* (Tassi) Goid; *Rhizoctonia* sp. and *Trichoderma* sp. (Table 3).

The BRS Tumucumaque cultivar showed the highest total number of colonies per seeds (420) in all the traditional varieties and biofortified cultivars obtaining the highest incidence of *Aspergillus niger* (41.66%); *Rhizopus stolonifer* (21.19%) and Yeast (1.92%) (Table 3).

Table 1. Mean values of water content (WC); germination (G); first seed count (FC); germination speed index (GSI); root length (RL) and aerial part length (APL) of the cowpea traditional and biofortified varieties.

VARIETIES						
TRADITIONAL	**WC (%)**	**G (%)**	**FC (%)**	**GSI**	**RL (cm)**	**APL (cm)**
Angelim	10.89 a	95.5 ab	88.0	11.83 c	13.09 a	9.74 ab
Mercado	11.22 a	95.0 ab	88.0	11.82 c	11.91 a	9.01 a
Manteiguinha	10.60 a	99.0 ab	97.0	12.35 c	12.30 a	11.88 b
BIOFORTIFIED						
BRS Aracê	13.20 b	100.0 a	97.0	16.57 a	12.10 a	12.11 b
BRS Xique-Xique	11.13 a	99.0 ab	100.0	16.32 a	13.00 a	15.48 c
BRS Tumucumaque	14.93 c	94.0 b	86.0	15.20 b	7.28 b	9.78 ab
CV (%)	1.61	2.46		4.77	12	9.84

Means followed by the same lowercase letter in the column do not differ statistically by the Tukey test at the level of 5% probability.

Table 2. Mean values for emergence (E); emergence speed index (ESI) and electrical conductivity (EC) of the traditional and biofortified cowpea varieties.

VARIETIES			
TRADITIONAL	**E (%)**	**ESI**	**EC (μS cm^{-1} g^{-1})**
Angelim	96 a	9.71 d	35.62 a
Mercado	99 a	10.59 cd	37.30 a
Manteiguinha	98.5 a	11.98 bc	37.32 a
BIOFORTIFIED			
BRS Aracê	98.5 a	14.67 a	49.77 b
BRS Xique-Xique	96.5 a	14.64 a	67.22 c
BRS Tumucumaque	86.5 b	12.36 b	77.12 d
CV (%)	2.69	5.95	4.39

Means followed by the same lowercase letter in the column do not differ statistically by the Tukey test at the level of 5% probability.

Table 3. Fungus incidence (INC) (%), contaminant total expressed in unit of colonies (UN) identified in traditional and biofortified cowpea varieties.

Incidence of fungi (%)	**VARIETIES**						
	Traditional			**Biofortified**			
	Angelim	**Mercado**	**Mant***	**BRS Aracê**	**BRS Xique-Xique**	**BRS Tumuc****	**INC Total (%)**
Aspergillus niger	15.56	19.62	28.9	30.93	29.91	41.66	166.58
Cladosporium herbarum	28.74	17.08	23.12	5.03	20.51	7.14	101.62
Penicillium sp.	26.94	22.15	38.72	25.17	13.67	28.09	154.74
Fusarium sp.	18.56	37.97	4.04	33.09	33.33	-	126.99
Phoma sp.	2.99	-	1.73	2.15	0.88	-	7.75
Rhizopus stolonifer	-	-	2.89	2.92	-	21.19	27.00
Macrophomina phaseolina	5.98	-	0.6	-	-	-	6.58
Trichoderma sp.	-	-	-	0.71	-	-	0.71
Levedura	-	-	-	-	1.7	1.92	2.41
*C. gloeosporioides****	1.23	-	-	-	-	-	1.23
Rhizoctonia sp.	-	3.18	-	-	-	-	3.18
Total species (UN)	7.00	5.00	7.00	7.00	6.00	5.00	
Total colonies (UN)	167.00	158.00	173.00	139.00	117.00	420.00	

*Manteiguinha. **BRS Tumucumaque. ****Colletotrichum gloeosporioides*.

4. Discussion

The water content in the cowpea seeds in the traditional varieties ranged from 10.6% to 11.62%, and in the biofortified varieties from 11.13% to 14.93%. Similar values (10.52% to 12.15%) were found for landrace cowpea varieties cultivated in Rio Grande do Norte (Silva et al., 2019). Bragantini (2005) reported that when the storage moisture of the bean is between 11% and 13%, the respiratory process is low, and thus seed deterioration is avoided and the physiological quality is maintained. According to Carvalho and Nakagawa (2012), storing seeds with 12-14% water content allows there to be seed degradation due to the respiration process that also favors the appearance of plant pathogen microorganisms. However, the seeds of the BRS Tumucumaque and BRS Aracê cultivars showed high germination power even with the 14.93% and 13.2% water content, respectively (Table 1). Considering our data, moisture did not interfere in the germination. These aspects are probably related to the time of harvest, processing and storage of the seeds.

The minimum 94% germination percentage observed in the traditional and biofortified varieties researched indicated that these seeds are within the standard determined by the Normative Instruction of the Ministry of Agriculture (Instrução Normativa do Ministério da Agricultura) (Brasil, 2013), that establishes identity and quality standards for the production and commercialization of seeds of the important agricultural crops, determining an 80% minimum germination percentage. The first count data showed that all the varieties and cultivars already presented germination of over 80% after the fifth counting day carried out in the tests. According to the Brazilian Association of Seeds and Seedlings (ABRASEM, 2017), all the varieties and cultivars assessed in the present research are within the 80% germination standard. These data showed the potential of the traditional and biofortified varieties studied.

Silva et al. (2019) obtained values for the germination results higher than the standard for three of the six landrace cowpea varieties studied. In the state of Ceará, Gomes et al. (2008) assessed the germinative potential of cowpea seeds produced and observed that all the seeds of twelve varieties were below the standard value.

The BRS Aracê and BRS Xique-Xique cultivars showed the highest germination speed index, 16.57 and 16.32, respectively. The germination speed index is based on the principle that seed batches that show faster germination speed are more vigorous.

Regarding the root length, the biofortified cultivars and traditional varieties showed 7.28 minimum and 13 cm maximum values. For the aerial part length, the smallest value was 9.01 cm while the biggest value was 15.48 cm. Our results showed higher values compared to the results of the studies by Silva et al. (2019) who observed a mean value of at most 4.8 cm for landrace cowpea varieties. These seedling growth parameters reflect in the field performance, as success at this development phase shows their productive potential.

All the varieties assessed in the present research showed very high vigor. These results were similar in part to those reported by Silva et al. (2019) in whose experiments only two cowpea varieties showed very high vigor, Pingo de Ouro and Sempre Verde with 92% and 86%, respectively. The high vigor values are related to the success of establishment and higher productivity in the field (Marcos Filho, 2015).

All the varieties showed high emergence value in the greenhouse. In the emergence test (E) and the emergence speed index (IVE) the faster seedling emergence is in the field, the more vigorous will be the seed sample/batch (Raisse et al., 2020).

The BRS Tumucumaque cultivar showed the highest electrical conductivity (CE) among all the varieties, with 77.12 µS cm-1 g-1, showing therefore a large quantity of leachates, since the higher the CE value, the higher the level of cell content extravasation to the seed imbibition process (Kruse et al., 2006). This data compared with the higher moisture value found, shows that this cultivar was stored under high moisture and was probably in the initial deterioration process, damaging the aerial part and the root, as these values for Tumucumaque were lower compared with the other cultivars and varieties. However, it was noted that even the varieties with higher CE values were not harmed in germination and emergence in the greenhouse and all the varieties showed full development.

Similar values were found in *Phaseolus vulgaris* L. (common bean) by Michels et al. (2014) with means ranging from 85.2 to 89.7 µS cm-1 g-1. However, our values surpassed in quality the landrace cowpea seeds studied by Silva et al. (2019), who obtained results ranging from 140 to 145 µS cm-1 g-1, these values represent double the quantity of leachates found in our sample with the highest value.

Regarding the health survey, the fungi that attacked the seeds can be divided into field or storage fungi. The field fungi generally remained quiescent during seed storage, while the storage fungi affected the stored seeds, because they can grow under relatively dry conditions (Galli et al., 2007). High incidence was found of *Aspergillus niger*, *Cladosporium herbarum*, *Penicillium* sp. in the traditional and biofortified varieties: 166.58%, 101.62% and 154.74%, respectively and are, according to Brasil (2009b), storage fungi. These results are similar to those reported by Gomes et al. (2008), Silva et al. (2019), Rodrigues and Menezes (2002), Silva et al. (2016) when they assessed seeds of cowpea varieties and observed predominance of *Aspergillus* sp. and *Penicillium* sp.

The *Aspergillus niger* fungus has occurred in bigger percentages than *Penicillum* sp. in other studies. According to Francisco and Usberti (2008) the larger incidence of *Aspergillus niger* compared to *Penicillum* sp. is because the *A. niger* fungus is a primary colonizer, in many cases associated with *Penicillum* sp., that occurs as secondary colonizer. Among the storage fungi, the lower occurrence of the *Cladosporium* sp. genus was observed by Ferreira et al. (2017), who reported a higher incidence of *Aspergillus* sp. compared to *Cladosporium* sp. on the surface of 'Red Mexican' bean seeds, corroborating the results in the present study.

Among the soil inhabiting fungi that infect cowpea, the presence was observed in the tested seed varieties of *Fusarium oxysporum* f. sp. *Tracheiphilum,* the causal agent of Fusarium Wilt, *Macrophomina phaseolina* the causal agent of grey stem rot and *Rhizoctonia solani* the causal agent of web blight.

The *Fusarium* sp. genus was present with high percentage incidence in a large part of the varieties, except for BRS Tumucumaque. This genus causes diseases that significantly damage the cowpea crop. The pathogen is a natural soil inhabitant, and survives as a saprophyte, normally transmitted in the seed interior and surface, from where the initial foci of the disease break out in cropped areas (Cardoso, 2000).

The BRS Tumucumaque cultivar showed the largest total number of colonies per seeds (420) among the traditional varieties and biofortified cultivars, obtaining the highest incidence for *Aspergillus niger* (41.66%); *Rhizopus stolonifer* (21.19%) and Yeast (1.92%). It is probable that the high water content (14.93%) favored the high incidence of contaminants in the seeds. According to Mantovani (2010), Carvalho and Nakagawa (2012) seeds with high water content become very vulnerable to colonization by large fungus and insect populations. However, it is pointed out that the diversity of fungus species found in BRS Tumucumaque was limited to five. In the Angelim, Manteguinha and BRS Aracê varieties seven different species were found, demonstrating that Tumucumaque showed higher incidence but in fewer fungus varieties.

Storage fungi, such as *Aspergillus* sp. and *Penicillium* sp., contaminate the grains after harvest and can live associated to the seeds even with lower moisture level (13% to 13.5%). Among the various fungi that attack stored grains, most start development when the moisture levels are above 13.5% (Fontes and Mantovani, 1993).

5. Conclusion

The traditional and biofortified varieties showed similar physiological and health quality. The water content present in the BRS Tumucumaque cultivar showed initial deterioration process, harming the aerial part and the root. Furthermore, it is probable that the bigger number of contaminating fungi found in this variety was related to the high water content present in the seeds. The biofortified cultivars can be indicated for planting in substitution for the traditional varieties.

Acknowledgements

The authors thank the Coordination of Improvement of Higher Level Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Cargill Foundation for financial support.

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