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Physiological and Sanitary Quality in Cowpea Seeds Produced in Rio Grande Do Norte, Brazil

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Abstract

The importance of cowpea (*Vigna unguiculata* (L.) Walp.) to the Northeast region of Brazil is undeniable, it is a major crop in local family agriculture. As a result, the research aimed to evaluate the physiological and sanitary quality of local varieties of cowpea coming from the state of Rio Grande do Norte. The experiment was carried out at the Seed Pathology and Seed Analysis Laboratory, in Federal University of Paraíba, Campus II, Areia, Paraiba, Brazil. Six cowpea varieties (Baeta, Coruja, Sempre Verde, Epace 10, Feijão da Bahia and Pingo de Ouro) from rural Area of the municipality of Apodi, Rio Grande do Norte, Brazil.were evaluated Seed physiological quality was evaluated by determining the first germination count (FGC), germination percentage (G%), seedling length (SL) and electrical conductivity (EC). The sanitary quality of the seeds was evaluated by the method of incubation in filter paper (blotter test). The experimental design was completely randomized, with 4 replicates. The Pingo de Ouro, Sempre Verde and Coruja varieties had germination percentages greater than the minimum standards required for commercialization, while the lower germination percentage and vigor of the Feijão da Bahia, Epace 10 and Baeta varieties may be associated with the presence of *Aspergillus* sp. and *Penicillium* sp.

Keywords: germination, fungal incidence, Creole seeds, *Vigna unguiculata* L.

1. Introduction

The cowpea (*Vigna unguiculata* (L.) Walp.), also known as string bean or Macassar bean is a legume predominantly cultivated by family farmers. It is considered as the staple food of the population, receiving great attention in the social and economic area because the low production cost and high nutritional value (Calvet et al., 2013; Fernandes et al., 2015). It develops satisfactorily in soils of low fertility (Silva, 2007), and considered moderately tolerant to salinity (Fageria et al., 2010). In Brazil, production in the year 2016/2017 was 713.3 thousand tons, and the production was mainly concentrated in the Northeast region. Planting starts from the first rainfall records, since the crop is large a fact that can also be attributed to its characteristics of water stress tolerance (CONAB, 2017).

The use of Creole seeds in agriculture is of extreme importance because of their high genetic diversity, which is fundamental for breeding programs, making it necessary to know the physiological potential of these varieties (Tsutsumi et al., 2015). In addition, it presents several advantages such as resistance to pests and diseases and good adaptation to local conditions as a result of the several cultivation generations carried out by the farmers, since these genotypes are influenced by environmental factors and consequently are endowed with greater greater genetic diveristy (Coelho et al., 2010).

The quality of the seeds used for planting is essential to guarantee crop establishment and good production (Tropaldi et al., 2010), ensuring uniformity in the plant stand and absence of diseases that are caused via seeds and consequently it will constitute vigorous plants (Souza et al., 2013). Usually, certain parameters are used to evaluate seed quality, these may include the seed's genetic, physical, physiological and sanitary nature (Marcos

Filho, 2015). From these parameters, the seed sanitary quality is highlighted, since it constitutes an efficient means of disseminating pathogens from a contaminated lot, the disease can be introduced into new areas, causing seedling death (Henning, 2005). The pathogens may be present on the surface, inside or mixed in the seed itself, presenting in various forms including spores to resistance structures (Santos et al., 2011). As a result, the research objective was to evaluate the physiological and sanitary quality of local cowpea varieties (*Vigna unguiculata* (L.) coming from the State of Rio Grande do Norte, Brazil.

2. Material and Methods

The work was developed at the Laboratories of Seed Pathology and Seed Analysis, in Federal University of Paraíba (UFPB), Campus II Areia, Paraíba, Brazil. Seeds of six varieties of cowpea (V_1: Baeta, V_2: Coruja, V_3: Sempre Verde, V_4: Epace 10, V_5: Feijão da Bahia, and V_6: Pingo de Ouro), from rural farmers of Córrego Site, Zona Rural of the Municipality of Apodi, Rio Grande do Norte, Brazil. After the harvest until conducting the experiment, the seeds were stored in pet bottles (polyethylene terephthalate) for a year, the same time equal to what farmers use, until new planting.

At the beginning, the seed water content was determined by the oven method at 105±3 °C for 24 hours (MAPA, 2009). Subsequently, physiological and sanitary quality tests were performed. For the evaluation of the physiological quality, the seeds were submitted to the germination and vigor tests according to the methodology described in the Rules for Seed Analysis (MAPA, 2009). The germination test was carried out using four replicates of 50 seeds for each variety, distributed under rolls of *germitest*® type paper moistened with distilled water 2.5 times the weight of the dry paper, placed at a temperature of 25±0.5 °C and 12-hour photoperiod. The percentage of germinated seeds (normal seedlings) was recorded on the fifth and ninth day after the test initiation (MAPA, 2009).

The vigor of the seeds was evaluated through the first germination count (FGC), performed on the fifth day after the germination test was installed and the result expressed as a percentage of normal seedlings. In addition it was classified into very high vigor: greater than 80%; high vigor: between 70 to 79%; average vigor: between 50 to 69%; low vigor: between 30 and 49%; and very low vigor: less than 29% (Vieira & Carvalho, 1994).

At the end of the germination test, seedling evaluation was carried out, measuring the length using a ruler graduated in centimeter of 20 random seedlings. The mean seedling length value was obtained by the arithmetic mean of the number of seedlings for each replicate, and the result expressed in cm.

For the electrical conductivity test (EC), four replicates of 50 seeds previously weighed and submitted to a plastic container containing 75 mL of distilled water and maintained in a germinator at 25 °C for 24 hours were used. After this period, the electrical conductivity was measured in the impregnation solution using a conductivi meter (Mars, model MB-11P), whose results were expressed in $\mu S\ cm^{-1}\ g^{-1}$.

The experimental design was completely randomized, with four replicates. Data were submitted to analysis of variance by the F test. The averages were compared by the Tukey test, at 5% probability using the Sisvar software (Ferreira, 2014).

The sanitary quality of the seeds was evaluated by the method of incubation on filter paper (Blotter test), using 200 seeds (ten replicates of 20 seeds) for each variety. The seeds were distributed in Petri dishes containing two sheets of filter paper (80 $g\ m^1$) previously sterilized and moistened with sterile distilled water (SDW) 2.5 times the weight of the dry paper. The plates were incubated at 20±2 °C and photoperiod for 12 hours. The quantitative and qualitative evaluations of the fungi associated with the seeds were carried out after seven days of incubation, and the seeds were examined individually under a stereoscopic microscope.

3. Results and Discussion

The water content of the seeds ranged from 10.52% to 12.15% in the Pingo de Ouro and Feijão da Bahia varieties, respectively (Table 1). These values are higher than those observed by Talamini et al. (2010) in which the physiological and sanitary quality of bean seeds (*Phaseolus vulgaris* L.) from family farmers in Sergipe, Brazil, obtained water content ranging from 8.6% to 10.70%. This difference can be related to the drying practice performed by the producers after the harvest, as well as by the climatic conditions of each region. However, this value is within the moisture pattern for bean seeds. According to Bragantini (2005), when the humidity of bean seeds is between 11% and 13%, the respiratory process remains low, prolonging the maintenance of seed quality.

Table 1. Moisture, vigor, germination, seedling length, and electrical conductivity in cowpea (*Vigna unguiculata* L.) varieties, produced in the state of Rio Grande do Norte

Varieties	Moisture content	Vigor	Germination	CP	EC
	------------------------------	% -----------------------------		------ cm ------	-- µS cm⁻¹ g⁻¹ --
Baeta	11.91	62 abc	67 b	4.5 abc	143.2 d
Coruja	11.94	71 ab	89 a	4.8 a	132.7 c
Sempre Verde	11.58	86 a	96 a	4.01 abc	106.7 b
Epace 10	11.88	32 d	67 b	3.99 bc	145.5 d
Feijão da Bahia	12.15	40 c	54 b	3.87 c	140.8 d
Pingo de Ouro	10.52	92 a	97 a	4.7 ab	65.33 a

Note. Means followed by the same letter in the column do not differ statistically by the Tukey test, at 5% probability.

The Pingo de Ouro and Sempre Verde varieties showed very high vigor 92% and 86% of normal seedlings at the first germination count, respectively. The Coruja variety has high vigor presenting 71% of normal seedlings. Mean vigor with 62% of normal seedlings is the Baeta variety. The varieties that presented low vigor were Feijão da Bahia and Epace 10 with 40% and 32% vigor, respectively (Table 1). These results differ from those observed by Silva et al. (2008), which evaluated the physiological and health quality of common bean (*P. vulgaris*) seeds from the State of Goiás, Brazil, observed low values of vigor for the varieties Aporé, Roxo 90, Corrente, Jalo Precoce and BRS Talisman, 21.09%, 7.43%, 9.93%, 17.23% and 33.23%, respectively. This difference among varieties may be associated with several factors. According to Carvalho & Nakagawa (2012), the physiological quality of the seeds can be influenced by the size of the seed, storage forms and conditions, sanitary conditions and physical and genetic constitution. It should be considered that the greater the seed vigor, the better the establishment of the field crop, increasing the stand uniformity and consequently, allowing a greater productivity (Moraes, 2006).

The germination values of the evaluated varieties ranged from (54% to 97%). The varieties with the greatest germination percentages were Pingo de Ouro, Sempre Verde and Coruja (97%, 96% and 89%), respectively (Table 1). These varieties are within the twinning standard proposed by the Brazilian Seed and Seed Association, which is 80% (ABSM, 2013). Coelho et al. (2010) obseved that *Phaseolus vulgaris* L. seed from the municipality of Lajes, State of Santa Catarina had germination values above 80%, thus showing the physiological potential of Creole seeds.

Feijão da Bahia (54%), Baeta and Epace 10 (67%) presented germination of less than 70% (Table 1). These results differ from those found by Gomes et al. (2008) who studied the physiological quality and the incidence of fungi in cowpea seeds produced in the State of Ceará. The germination for Sempre Verde cultivar was 70.33% and other cultivars (Epace 10, Canapu, Costela de vaca, Lizão 1, Lizão 2, Corujinha, Parambú, Galanjão, Pingo de 1, Pingo de ouro 2 and Pingo de ouro 3) had germination percentages less than this value. Marques et al. (2006) evaluated the physiological quality of common bean seeds, with a mean of 64% in the Pérola cultivar produced in Minas Gerais and 62% in the Talismã cultivar, produced in the State of Goiás, evaluating the germination of six cultivars of cowpea from the municipalities of Ceará, found germination percentage between 69% and 88%.

Marques et al. (2006) determined that the germination of six cultivars of cowpea from the municipalities of Ceará werr between 69% and 88%, compared to 64% for common bean seeds from a Pérola cultivar produced in Minas Gerais and 62% for a Talismã cultivar produced in the State of Goiás,

These differences in germination are probably due to the different seed storage systems used by the farmers after harvest until planting. The farmers may use anything from PER bottles to zinc silos for seed storage, and, therefore, they may not provide an ideal environment for seed storage, resulting in decreased seed germination. Without the ideal storage environment, the seed may be exposed to high humidity and determinal temperatures, which when combined with the type of packaging used (generally permeable or semipermeable) has a great influence seed conservation and reduced germination (Antonello et al., 2009). Differences in physiological quality between varieties and seed lots can be attributed not only to the genotype but also to the effects of environmental conditions prevailing during the maturation and harvesting phase (Aguero et al., 1997).

According to Silva et al. (2012), factors such as environmental conditions field seed development, harvest, drying, processing, and storage affect the seed quality. Rapid and uniform germination is important in the semi-arid conditions, since water resources are limiting, therefore, the use of a vigorous plant material would minimize the abiotic stresses and decrease the exposure time to the pathogens responsible for the initial infection of seedlings. For the seedling length, the greatest results were observed in the Coruja variety (4.8 cm), followed by the Pingo de Ouro and Baeta (4.7 and 4.5 cm), respectively, whereas the shorter length was observed with the Feijão da Bahia (3.87 cm) seeds. Evaluating the physiological quality in seeds of common bean varieties cultivated in Vitória da Conquista, State of Bahia, Araújo Neto et al. (2014) also observed significant differences in the seedling lengths of the varieties used, where Carioca L1 varieties increased seedling lengths t (6.4 cm) compared to Rosinha shorter seedling length (4.96 cm). Vigorous seedlings give rise to productive plants.

The varieties Epace 10, Baeta and Feijão da Bahia had a greater release of electrolytes during seed imbibition, resulting in higher values of electrical conductivity (145.5, 143.2 and 140.8 µS cm^{-1} g^{-1}, respectively) in relation to the others. While the lowest conductivities were observed in Pingo de Ouro, Sempre Verde and Coruja (65.33, 106.7 and 132.7 µS cm^{-1} g^{-1}, respectively) showed greater vigor. This is because the lower the electrical conductivity value correlates to greater membrane integrity and consequently increased seed vigor. The electrical conductivity test can detect the first symptoms of seed deterioration, since it is related to the integrity of cell membranes (Albuquerque et al., 2001; Muasya et al., 2002). These results are consistent with Michels et al. (2014), who studied the physiological quality of bean seeds produced in the western Plateau of Santa Catarina, who was able to classify the varieties in two or more classes regarding the release of solutes, observing that the creole varieties formed the class with lower release of solutes.

In the sanitary analysis of the varieties of cowpea, from farmers of Apodi, Rio Grande do Norte, the presence of phytopathogenic fungi and storage were verified. Similar results were observed by Barreto et al. (2006) who evaluated the health of macassar bean seeds from the municipality of Pombal, State of Paraíba. They found the following pathogens: *Aspergillus flavus* (28.7%), *Aspergillus niger* (20%) and *Fusarium* spp. (14.6%), *Penicillium* spp. (12.6%), *Cladosporium* sp. (7.3%), *Chaetomium* sp. (4.6%), *Botrytis* sp. (3.7%), *Periconia* sp. (2.8%) and *Rhizopus* (1.8%). Silva et al. (2016) evaluating the incidence of pathogens in bean seeds from 37 municipalities of Rio Grande do Norte, found in 94% of the seed samples, *Aspergillus* spp. (60%), *Bipolaris* sp. (8%), and *Beryllium* spp. *Colletotrichum* spp. (5%), *Fusarium* spp. (53%), *Macrophomina* sp. (60%), *Penicillium* sp. (13%), *Pleurophagminum* sp. sp. (70%), bacteria of the genera *Rhizobium* sp. (100%) and *Pantoea* sp. (3%), the pest *Callosobruchus maculatus*.

The literature has shown that storage fungi, especially *Aspergillus* and *Penicillium* sp. (Singh et al., 2007; Biemond et al., 2013) cause most of the cowpea seed deterioration during storage. In the present study it was observed that there were differences between the varieties regarding the incidence of *Aspergilus* sp., where Epace 10, Baeta and Feijão da Bahia obtained the greatest incidence of this pathogen with 24, 21.75 and 20%, respectively (Figure 1A). In addition, the Coruja and Pingo de Ouro varieties had a low incidence (less than 10%) of the pathogen. The presence of *Aspergilus* sp. may cause reduction in seed germination, especially in those stored with high water content, when this fungus is present in large quantities. These results differ from those observed by Gomes et al. (2008), in which analyzing the physiological quality and incidence of fungi in cowpea beans produced in the State of Ceará, observed a high incidence of *Aspergilus* in the Pingo de Ouro variety.

The varieties studied also differed in the incidence of *Penicillium* sp., where Baeta, Epace 10 and Coruja had higher infestations, 14.5%, 14% and 11.5%, respectively (Figure 1B). Silva et al. (2016) also observed the presence of these pathogens in the cowpea varieties from the municipalities of Rio Grande do Norte. These fungi are highly harmful to seeds, reducing germination and causing embryo death, rotting seeds, mass heating with the acceleration of the respiratory rate and its consequent deterioration, besides promoting the production of mycotoxins such as aflatoxin, which is lethal to man and animals (Carvalho & Nakagawa, 2012). This fact was observed in our research, since the varieties (Baeta, Epace 10 and Feijão da Bahia) that had a higher incidence of these pathogens (Figures 1A and 1B) also presented low germination (Table 1).

In the present study, the presence of *Fusarium* sp. was greater in the Epace 10, Sempre Verde and Baeta varieties (8.5, 8.5 and 8% incidence), respectively (Figure 1C). This fungus has been reported in some sanitary cowpea surveys (Ikran & Dawar, 2013; Ito et al., 2013). Some Fusarium genera (*Fusarium oxysporum* and *Fusarium solani*) are important pathogens for bean culture, being responsible for diseases such as fusariosis, cervix and root rot, and therefore being highly harmful and can drastically reduce yields (Silva et al., 2014). These pathogens not only possess high pathogenicity but can be transmitted by seeds and have the capacity to survive in the soil, even in the absence of the specific host (Sallis, 2001). Silva et al. (2002) reported finding fusarium species inhibited cowpea germination of seeds from Serra Talhada and Caruaru, PE, and observed fungus growth on the cotyledons and leaves, and root necrosis on the seed that did germinate.

The Baeta did not present an incidence of *Cladosporium* sp., with a percentage of less than 1.5% of incidence observed for Coruja, Sempre Verde and Pingo de Ouro. The varieties Epace 10 and Feijão da Bahia had a higher infectious incidence,10% and 4%, respectively (Figure 1D), which corroborate the results obtained by Guimarães and Carvalho (2014), who observed *Cladosporium* sp. in common bean seeds cv. 'Pérola'. Pérola had spots or greenish growths on the seed surfaces, mainly in the zone corresponding to the embryo, more precisely in the seed thread, and 59.5% of the seeds were infected.

The genus *Rhizopus* sp. has little economic importance in respect to seeds, however, it may hinder the detection of other pathogens, because its rapid growth may cover the seeds (Torres & Bringel, 2005). In the our research, the presence of this fungus was detected in the Baeta (10%), Coruja (2.5%), Sempre Verde and Pingo de Ouro (0.5%) varieties. Epace 10 and Feijão da Bahia had no incidence of this pathogen (Figure 1E). These results differ from those observed by Silva et al. (2008) in which the genus *Rhizopus* sp. was also detected in all cultivars, with a higher incidence for the cultivars Sempre Verde and Pingo de Ouro (5%). Barreto et al. (2016) evaluating the incidence of pathogens in (*Gossypium hirsutum* L.) treated with agave (*Agave angustifolia*) strata also identified this fungus.

The pathogens *Botrytis* sp. and *Chaetomium* sp. were found in the seeds, but with a low percentage of contamination (less than 3%). The owl variety had a 1.5% incidence of *Botrytis* sp. (Figure 1F). Silva et al. (2008) also observed a low incidence of this pathogen in cowpea beans from Goiás. Other authors have also reported the presence of this fungus, such as Araújo et al. (2013) in seeds of Aroeira (*Astronium urundeuva*) and Maciel et al. (2012) in Angico Vermeho (*Parapiptadenia rigida*). Regarding the fungus *Chaetomium* sp., the highest incidence was recorded in the variety Sempre Verde 5% (Figure 1G). Similar to the results found by Barreto et al. (2015) that also verified a low incidence (4.6%) of this fungus in cowpea (*V. unguiculata*). Kobayasti et al. (2011) found that the seed deterioration the smooth top (*Jatropha curcas* L.) is related to the presence of *Chaetomium* sp. The action of this pathogen depends on the physical and physiological conditions of the seeds and the environmental factors that predominate during the storage (Ruiz Filho et al., 2004).

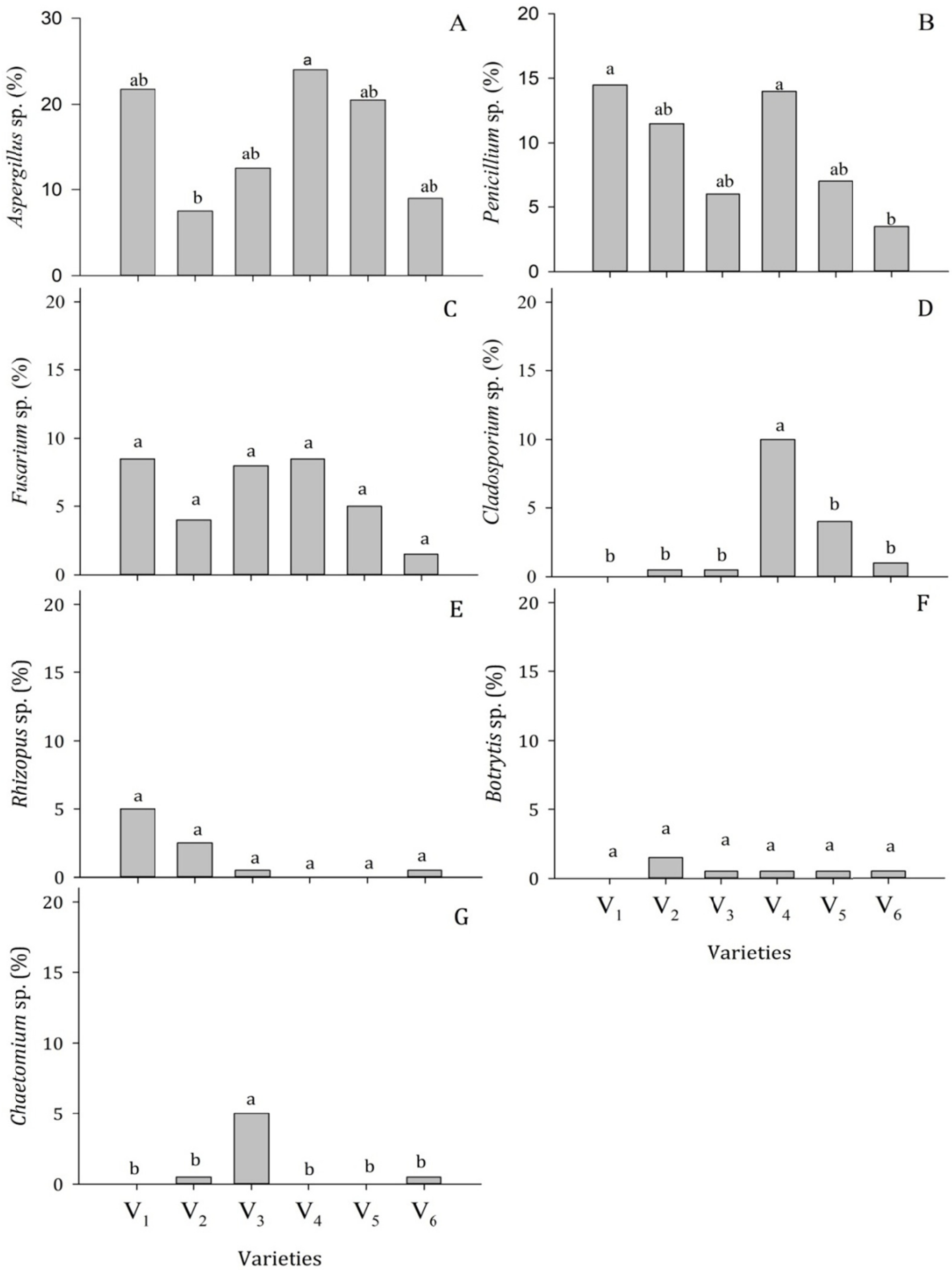

Figure 1. Incidence of fungi on seeds of cowpea (*Vigna unguiculata* L.) produced in the state of Rio Grande do Norte

4. Conclusions

The creole cowpea varieties Pingo de Ouro, Sempre Verde and Coruja had germination percentages and vigor above the minimum standards required for commercialization, and recommended for planting. The low germination percentage and vigor of the varieties Feijão da Bahia, Epace 10 and Baeta, may be associated to the presence of *Aspergillus* sp. and *Penicillium* sp.

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