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Physiological and phytosanitary quality of common bean seeds used by small growers in Minas Gerais state, Brazil

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Abstract

The physiological and phytosanitary qualities of common bean seeds used on a small farming system depend on the cultural practices, drying system and storage conditions employed. The objective of this work was to evaluate eight lots of common bean seeds using germination, vigor and phytosanitary tests. The lots were produced in 2005 and 2006 in "Zona da Mata", Minas Gerais State, Brazil, and analyzed for moisture content, germination quality, first germination count, seedling vigor, seedling emergence, electric conductivity, seedling dry weight, seedling length, hypochlorite test and blotter test. The experimental design was completely randomized with four replications. The mean seed moisture content from all lots was 8.4 %. Four lots were classified as high vigor, one as intermediate vigor and three lots as low vigor. The first germination count and electrical conductivity tests were the best for evaluating bean seed physiological quality. All seed lots were infested by fungi, mainly *Alternaria*, *Cladosporium* and *Fusarium*.

Keywords: *Phaseolus vulgaris* L. Pathology seed. Production.

Introduction

Brazil is the world's largest common bean (*Phaseolus vulgaris* L.) producer and the cultivation of this legume is widespread across the country (BRASIL, 2013). Common bean is among the ten most cultivated crops and is a traditional food in Brazil. The annual production is 2,564,790 metric tons harvested from 1,895,267 ha in 2013 for an average yield of 1,353 kg ha^{-1} (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA, 2014). Only about 10 % of small landholders in Brazil use certified seed and the vast majority rely on their own saved seed from yearly harvests. The lack of certified seed use is justified partly by the cost and a lack of awareness of the importance of using seeds with good phytosanitary and physiological quality every year (MENTEN et al., 2006).

The fungal diseases of common bean during field culture and under storage conditions are the main reasons to preserve seed health and viability. Whereas *Penicillium spp*. and *Aspergillus spp*. are the predominant pathogens with the highest incidence in the field, other pathogens such as

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Alternaria spp., *Fusarium spp*., *Rhizoctonia spp*. have also been reported (FRANCISCO; USBERTI, 2008). The seed vigor tests based on the seedling growth and electrical conductivity have been used to select the different vigor levels in bean seed lots (ARAUJO, et al., 2011; CARVALHO; NAKAGAWA, 2012; LOBO JUNIOR et al., 2013). This study aimed to evaluate the physiological and phytosanitary quality of different lots of common bean seeds used by small-scale farmers in Brazil.

Material and methods

The experiment was carried out at the Laboratory of Seed Quality Control, Department of Crop Science, Universidade Federal Rural do Rio de Janeiro. Samples from eight seed lots produced by small farmers in Zona da Mata, Minas Gerais, were used. These lots have been used by farmers who reserve their own seeds or get them from other farmers in exchange for other products. Often the choice of seeds is exclusively based on the type of grain or seed coat color and taste, independent of physiological quality and harvest time. The conservation of these seed lots was carried out without seed moisture content control and there was no seed treatment on different packages and environments. The lots were identified based on seed coat color and harvest date; Red Coat: R1, R2, R3, R7 (harvested in April 2006); Red Coat: R4, R5, R6; Black Coat: B (harvested in May 2005). To evaluate seed physiological quality, subsamples from each lot were examined for the following parameters, following the national rules for seed testing (BRASIL, 2009): weight of 1.000 seeds (g), which was obtained by the mean weight of eight replicates of 100 seeds each; moisture content based on drying seeds at 105 ºC for 24 hours; percentage of germination assessed with 50 seeds in a rolled paper towel and kept at alternating temperatures and light of 20 °C per 16 h and 30 °C per 8 h in a precision incubator (Electrolab model EL 202). Evaluations were performed after 5 and 9 days, and results presented as a percentage of normal seedlings; first count of germination was based on the normal seedlings in the first assessment of germination test on day 5; percentage of strong seedlings at which normal seedlings were $\geq$ 3.0 cm long with “strong” hypocotyls. Seedling emergence (SE) was analyzed based on 100 seeds placed on washed and sterilized sand irrigated daily, and kept at 70 % of water holding capacity. It was evaluated at the opening of the first pair of leaflets at 5 (SE5), 7 (SE7) and 9 days (SE9) after sowing. Electrical conductivity was measured with a conductivity meter (DIGIMED model DM-31) using 50 seeds that were weighed, immersed in 75 ml of distilled water and incubated at 25 °C/24 hours; the results are expressed in $\mu s.cm^{-1}.g^{-1}$. For seedling length (mm), 20 seeds were distributed on two parallel lines on paper and kept in the incubator with alternating temperature and light as above; the evaluations were performed 7 days after the start of the test, by measuring the length from the hypocotyl to the apex of normal seedlings. Seedlings were then cut from the storage tissues, dried in an oven at 60 ºC for 24 hours, and mean seedling dry weight for each replicate was determined. The hypochlorite test was conducted by soaking 50 seeds in a solution of sodium hypochlorite 5 % for 5 minutes. The seeds were individually examined, determining the percentage of wrinkled and not wrinkled seeds. The phytosanitary test was carried out following the method of Torres and Bringel (2005), using for each lot 20 seed sub-samples, totaling 400 that were distributed in boxes containing two sheets of sterilized and moistened filter paper with sterilized distilled water. The boxes were then placed in a growth chamber at 20 ± 2 °C with 2.000 lux for 12 hours light and 12 hours dark for 7 d when developing pathogens were identified (NEERGAARD, 1979). Seeds were individually examined under a stereo microscope at 20 x (Olympus CX41). The fungal genus was identified based on its morphological characteristics, following authentic taxonomic keys and results were expressed in percentages.

The experimental design was completely randomized design with four replicates. Data analysis was performed using one-way ANOVA followed by Tukey's test ($P < 0.05$). For the analysis of germination, first count and seedling emergence, the values were converted in arc sin √ x/100 before analysis of variance, and original means are presented in the tables.

Results and discussion

The mean seed moisture content was 8.4 % and ranged from 8.1 to 8.7 %. No significant differences among lots were observed. Seed lots R1, R4, R5 and R6 had the greatest weight of 1.000 seeds and lot R2 had the lowest value; seed lots R3, R7 and B were intermediate for 1.000 seeds weight (Table 1).

Table 1. Electrical conductivity in $\mu s.cm^{-1}.g^{-1}$ (EC), seedling emergence (%) at 5, 7 and 9 days (SE5; SE7 and SE9), germination (G), normal seedlings first count germination (FCG), normal strong seedlings (NSS) and seedling length (SL), seedling dry matter (SDM), weight of 1,000 seeds (W1000), hypochlorite test (HP) and seed moisture content (SMC) of eight common bean (*Phaseolus vulgaris* L.) seed lots *.

	SEED LOTS							
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**B**
EC	88.7 e	129.0 d	94.2 e	125.9 d	249.2 b	312.6 a	107.7 e	176.6 c
SE5	84 a	72 b	73 b	0 c	2 c	2 c	73 b	0 c
SE7	92 a	94 a	89 a	48 b	42 b	5 c	96 a	39 b
SE9	95 a	94 a	92 a	66 b	43 c	5 d	96 a	63 b
G	99 a	90 b	99 a	75 c	40 e	9 f	98 a	68 d
FCG	70 a	72 a	82 a	20 b	23 b	4 c	71 a	27 b
NSS (%)	35 a	39 a	39 a	0 b	3 b	0 b	39 a	1 b
SL (cm)	20.3 a	22.5 a	17.5 b	12.0 d	14.0 c	10.5 d	21.0 a	11.4 d
SDM (g)	3.3 a	2.2 bc	2.5 abc	2.4 bc	1.4 d	0.3 e	2.9 ab	2.0 cd
W1000 (g)	241 ab	174 d	216 c	247 a	240 ab	250 a	217 c	227 bc
HP (%)	84 bc	77 c	90 ab	89 ab	53 d	48 d	94 ab	98 a
SMC (%)	8.7 a	8.1 a	8.5 a	8.4 a	8.3 a	8.3 a	8.1 a	8.7 a

*Means followed by same letter, in the line, aren't significantly different according to Tukey test ($P < 0.05$).
Source: Elaborated by the authors (2017).

Lots R1, R3 and R7 showed the highest germination ranging between 98 % and 99 %. Lots R4, R5, R6 and B germinated below the minimum standard of 80 % established for this species, based on standards for certified seed (BRASIL, 2013). Seeds from R5 and R6 lots showed the lowest germination (40 % and 9 %, respectively). The lots with the highest and lowest values of seedling emergence on sand were the same as the ones observed on the germination test (Table 1).

First count of germination showed the R1, R2, R3 and R7 lots as being the most vigorous. The same result could be seen using the "strong seedlings" evaluation 5 days after the beginning of the test (Table 1). The electrical conductivity (EC) test indicated R1 and R3 lots as having the highest vigor and R5 and R6 as having the lowest vigor seed lots. These results coincide with the germination values of these seed lots. The EC values between 88.7 and 107.7 $\mu s.cm^{-1}.g^{-1}$ were associated with high vigor lots and values over 176.6 μs.cm-1.g-1 were linked with less vigor and viability, suggesting limitations associated with the use of these seeds for sowing (Table 1). The EC test for bean seeds could identify the best cell membrane integrity resulting in the lowest release of solutes, showing a negative correlation to the germination and primary root length (COELHO et al., 2010).

Vigor tests could help on making decisions through a quality control program in seeds such as the EC test used for soybean and pea, and could be extended to evaluate the physiological quality of bean seeds (CUSTÓDIO, 2005).

The electrical conductivity test in mung bean seeds have shown high correlation with seed aging test and field seedlings emergence (ARAÚJO et al., 2011). The results of this study have identified the EC test as a promising tool for determining and classifying vigor among bean common seed lots.

Seedling emergence data have confirmed R1, R2, R3 and R7 as the superior lots. The highest seedling dry weight values were found for R1, R3 and R7 (Table 1). The large percent (48-98%) of damaged seeds in hypochlorite test indicated substantial mechanical damage of the tegument, which might hinder the absorption of water and, hence, the germination.

Laboratory results suggested that bean seed lots such as R4, R5, R6 and B harvested in 2005 may have more advanced stages of deterioration which can result in lower plant populations and imposing serious losses on productivity and increasing production costs per unit area. Although the test results demonstrate the poor outcomes from R5 and R6 lots, in some cases the farmers have used those causing losses in the crop establishment.

Seed lots harvested in 2006 showed higher germination and minor deterioration values compared to those from 2005. The effects of storage conditions are also evident, since the farmers store seeds in containers and locations exposed to variations of temperature and relative humidity. According to Barros (2007), the storage of seeds on small farms has been problematic due to the frequent lack of minimum infrastructure for seed conservation. Storage under unsuitable conditions has contributed to common bean seed quality reduction, negatively affecting crop establishment, harvesting and final production. After harvesting and processing, bean seeds should be stored at 12 % or less seed moisture content. Control of insects and insects such as beetles, weevils and moths should be also constant (MENTEN et al., 2006).

It was observed contamination by several fungi in all bean seed lots, including species of *Alternaria, Cladosporium, Fusarium, Aspergillus, Penicillium, Colletotrichum* and *Curvularia* (Table 2). These pathogens may have contributed to germination reductions on the seed lots. The Ministry of Agriculture Livestock and Supply in Brazil has established tolerance levels for seed diseases in *P. vulgaris* as 3 % anthracnose (*Colletotrichum lindemuthianum*), 2 % bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) and 0 % white mold (*Sclerotinia sclerotiorum*) (BRASIL, 2005). This agency has proposed “zero tolerance” for *Colletotrichum lindemuthianum*, *Fusarium oxysporum f.sp. phaseoli*, *F. solani f.sp*. *phaseoli, Sclerotinia sclerotiorum* and *Xanthomonas axonopodis* pv. *phaseoli*. For these pathogens, the use of healthy or adequately treated seeds has been the main control measure for the integrated management of diseases (MENTEN et al. 2006). In this research, lot R6 infected by *Colletotrichum spp*. and all lots other than R6 infected by *Fusarium spp*. would probably not be approved for marketing and multiplication on the national system for certified seeds.

Table 2. Percentage of pathogen occurrence on eight common bean (*Phaseolus vulgaris* L.) seed lots utilized by small farmers in Minas Gerais State, Brazil.

	SEED LOTS									
Pathogen	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**B**	**Number of contamined Lots**	**Total (%)**
	Contaminant (%)									
Alternaria spp.	15	0	45	0	5	0	15	5	5	85
Cladosporium spp.	10	15	0	35	0	0	0	0	3	60
Fusarium spp.	5	5	15	25	30	0	5	5	7	90
Aspergillus spp.	0	0	0	5	10	0	5	5	4	25
Curvularia spp.	0	0	0	5	0	0	0	0	1	5
Penicilium spp.	0	0	0	5	5	0	0	0	2	10
Colletotrichum spp.	0	0	0	0	0	5	0	0	1	5
Total (%)	30	20	60	75	50	5	25	15		
Number of pathogens	3	2	2	5	4	1	3	3		

Source: Elaborated by the authors (2017).

Torres and Bringel (2005), working with five macassar bean seeds, observed the highest incidence of *Aspergillus niger* and *Aspergillus flavus*. The authors also observed that accelerated aging and electrical conductivity test were the most efficient for the classification of seed lots at different levels of vigor, demonstrating that even lots of seeds with high viability and vigor could spread pathogens and affect the productivity.

The enzymes action and toxins produced from microorganisms as fungi cause cell wall degradation in plant tissues (HENNING et al., 2009). *Aspergillus ochraceus* on soybean seed surface caused high seed tissues degradation and reduced the seed vigor (ROCHA et al., 2014). The seeds are an effective source for dissemination and transmission of pathogens, often introducing new pathogens into disease free areas, and reducing the physiological quality of seed lots from these areas as well (NEERGAARD, 1979). For post-harvesting management, the seed should be dried until the levels of water content (10-12% SMC) inhibiting fungi growth. Seed cleaning and classification associated with a suitable environment for storage (percentage relative humidity + temperature $^{o}C \leq 55.5$) could minimize the outbreak of diseases treatment.

Seed storage temperature below 25 ^{o}C and 13.0 % seed moisture content appear suitable to preserve common bean seed in relation to viability and health, up to an 8-month period (FRANCISCO; USBERTI, 2008). However, in this experiment the seed lots were harvested and stored for more than one year in ambient conditions with temperature and relative humidity variables. Investment is necessary to improve the quality of seeds especially upon disease control during the bean cultivation and seed conditioning and storage.

Conclusion

Four lots were classified as 'high vigor', one as 'intermediate vigor' and three as 'low vigor'.

The first count germination and electrical conductivity tests were the best for evaluating bean seed physiological quality.

All seed lots were infested by fungi, mainly by *Alternaria spp*., *Aspergillus spp*; *Cladosporium spp*. and *Fusarium spp*.

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Qualidade fisiológica e fitossanitária de sementes de feijão utilizadas por pequenos agricultores em Minas Gerais, Brasil

Resumo

A qualidade fisiológica e fitossanitária de sementes de feijões usadas em um pequeno sistema de produção depende das práticas culturais, sistema de secagem e condições de conservação empregadas. Desta forma, objetiva-se com este trabalho avaliar a qualidade de oito lotes de sementes de feijão usando os testes de germinação, vigor e fitossanitário. Os lotes de sementes foram produzidos em 2005 e 2006 por agricultores da zona da mata do Estado de Minas Gerais, Brasil. As sementes foram analisadas quanto ao teor de água, porcentagem de germinação, primeira contagem de germinação, classificação do vigor, emergência de plântulas, condutividade elétrica, peso seco de plântulas, comprimento de plântulas, teste de hipoclorito e "blotter test". O delineamento experimental foi inteiramente casualizado, com quatro repetições. O teor de água médio de todos os lotes foi de 8,4 %. Quatro lotes foram classificados como alto vigor, um de vigor intermediário e três lotes de baixo vigor. A primeira contagem do teste de germinação e condutividade elétrica foram os melhores testes para a avaliação da qualidade fisiológica de sementes de feijão. Todos os lotes de sementes estavam infestados por fungos, principalmente *Alternaria, Cladosporium e Fusarium*.

Palavras-chave: *Phaseolus vulgaris* L. Patologia de sementes. Produção.

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