Australian Journal of Crop Science
AJCS

AJCS 13(02):300-308 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.02.p1466

Common bean seed quality as affected by cover crop mixtures and nitrogen fertilization

Alexandre Pedrinho[1,3*], Eduardo Mariano[2], Luis Fernando Merloti[1,3], Marco Eustáquio de Sá[3]

[1]Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil
[2]College of Agricultural Sciences, São Paulo State University, Botucatu, SP, Brazil
[3]College of Natural Sciences and Engineering, São Paulo State University, Ilha Solteira, SP, Brazil

***Corresponding author: alpedrinho@usp.br**

Abstract

Several studies have shown that cover crops can increase common bean (*Phaseolus vulgaris* L.) yield. However, little is known about the influence of cover crop mixtures in association with topdressing N fertilization on common bean seed quality. We, therefore, evaluated the physiological quality of common bean seeds produced under cover crop residues and N- fertilized in a no-till farming system. A 5 × 4 factorial experiment (cover crops and N rates) was performed under field conditions in Mato Grosso do Sul, Brazil. Single-specie and cover crops mixtures [pear millet (*Pennisetum glaucum*), pear millet-jack bean (*P. glaucum*; *Canavalia ensiformis*), pear millet-pigeon pea (*P. glaucum*; *Cajanus cajan*), pear millet-sunn hemp (*P. glaucum*; *Crotalaria juncea*), and pear millet-velvet bean (*P. glaucum*; *Mucuna pruriens*)] were grown and common bean was planted in succession. Nitrogen fertilizer (50, 100, and 150 kg N ha^{-1}) was topdressed applied to common bean at the V4 stage. At maturity, common bean plants were harvested, and physiological quality of seeds was assessed. The first germination count, final germination, germination speed index, accelerated aging, and N and protein concentration were influenced by cover crop residues and/or N rates (main effect). A cover crop × N rate interaction was observed for seedling dry biomass after germination and accelerated aging. All treatments produced seeds with high physiological quality. Although the cover crops showed variable results, the application of 100 kg N ha^{-1} in topdressing slightly improved seed quality attributes compared to the other N rate treatments. We conclude that addition of N fertilizer and cover crop treatments does not affect on common bean seed quality to a large extent.

Keywords: legume; nitrogen fertilizer; pear millet; *Phaseolus vulgaris* L.; physiological quality.

Abbreviations: CEC_cation exchange capacity; DAE_days after emergence; DAI_ days after incubation; H+Al_potential acidity; PM_pear millet; PM-JB pear millet-jack bean; PM-PP_pear millet-pigeon pea; PM-SH_pear millet-sunn hemp; PM-VB_pear millet-velvet bean.

Introduction

The common bean (*Phaseolus vulgaris* L.) is one of the most important legumes for human diet, due to its high nutritional quality (20-25% protein), vitamins, and minerals (Broughton et al., 2003). Brazil is considered one of the largest producers worldwide of this legume (Zucareli et al. 2016). Low inputs requirement, high economic value, and adequate climate conditions enabling production over three growing seasons throughout the year (Aug.-Nov., Dec.-Mar., and Apr.-Jul.). In 2017, Brazilian common bean production reached approximately 3.4 million Mg, with yields averaging only 1.0 Mg ha^{-1} (CONAB, 2018). These low yields are due to a large variety of systems used for common bean production. From subsistence crops (representing 33% of the national production, characterized by farms with <10 ha in size, low or no fertilizer input, and yields lower than 0.9 Mg ha^{-1}) to technically highly advanced crops (with large farms <100 ha in size under no-till agricultural practices, high input of fertilizers, and high-yielding levels >3.0 Mg ha^{-1}). This relatively large yield gap is also explained by biotic and abiotic factors including drought, weeds, pests, and mainly to the use of low quality seeds (Broughton et al., 2003).

A feasible alternative to reduce the yield gap in these areas is the adoption of high quality seeds, which have resulted in good agronomic performance and consequently high yields. High quality seeds possess great genetic, physical, physiological, and sanitary attributes (Zucareli et al., 2016), which helps crops withstand environmental stresses and defend against pests and diseases. Among those attributes, physiological quality is one of the most important and is related to the ability that seeds have in performing vital functions. Thus, seeds with high physiological quality promote fast germination, uniform emergence, and yielding seedlings with great tolerance to environmental adversity (França Neto et al., 2010; Marcos Filho, 2005; Zucareli et al., 2016). In general, physiological attributes of seeds are highly influenced by the environment (*i.e.*, climate conditions) in which the seeds were produced (Zucareli et al., 2016) and by crop management practices, such as the use of cover crops and fertilizers, which may affect the nutritional and sanity status of the parent plant.

The use of cover crops has attracted the interest of common bean growers due to the potential benefits on seed yield and sustainability of the agroecosystem (Decker et al, 1994). Most studies have shown that cover crops can increase organic C content, nutrient availability, water infiltration and storage, weed control, and microbial activity (Teasdale, 1996; Fageria et al., 2005). Additionally, cover cropping can decrease nutrient loss and soil temperature (Nielsen et al., 2015). However, little is known about the influence of cover crops, primarily grass-legume mixtures, on the physiological quality of common bean seeds. To the best of our knowledge, only two studies have assessed the influence of cover crops on common bean seed quality (Abrantes et al., 2015; Silva et al., 2017). In addition, topdressing N fertilization is a widespread practice to sustain high yields of common bean. The biological N fixation does not supply adequate amount of N to bean plants (Coelho et al., 1998; Silva et al., 2004). Furthermore, several researchers have reported that N fertilization improves nutritional and yield parameters of common bean (Silva et al., 2004; Amaral et al., 2016). However, there is still a great lack and conflict of information related to the amount of N provided on the physiological aspects of the common bean seeds.

Thus, the objective of this study was to evaluate the physiological quality of common bean seeds produced under cover crop straw (grass and grass-legume mixtures) and N-fertilized in a no-till cropping system. We hypothesized that common bean plants grown under residues of cover crop mixtures and high N inputs will produce seeds with higher physiological quality than seeds produced from plants grown under single-specie cover crop and without N fertilization.

Results

Physiological quality of seeds

The results of variance analysis are shown in Table 1. The common bean first germination counting, final germination, accelerated aging, and germination speed index exhibited similar patterns (Fig 1). In all tests, common bean seed quality was influenced by cover crops and N fertilization (main effect). In the first germination counting, common bean plants under pear millet-jack bean (*Pennisetum glaucum; Canavalia ensiformi*; PM-JB) mixture produced seeds with higher vigor than the remaining treatments (Fig 1a). Similarly, common bean plants under PM-JB produced seeds with a greater value of final germination and germination speed index compared to pear millet-sunn hemp (*P. glaucum*; *Crotalaria juncea*; PM-SH), which had the lowest rate (Fig 1bd). For the accelerated aging test, common bean grown under PM-JB mixture produced seeds with greater vigor than seeds from PM-SH and pear millet-velvet bean (*P. glaucum*; *Mucuna pruriens*; PM-VB; Fig 1c). The application of 100 kg N ha^{-1} resulted in higher germination of common bean seeds compared to all other treatments (Fig 1b). The addition of 100 N kg ha^{-1} resulted in superior final germination count, accelerated aging, and germination speed index in comparison to 50 kg ha^{-1} and the control (Fig 1acd). Common bean plants grown under pear millet-pigeon pea (*P. glaucum*; *Cajanus cajan*; PM-PP) and PM-SH produced seeds with lower electrical conductivity (i.e., less leakage of cell content) values than those produced under PM-JB and PM-VB (Fig 1e). The highest N rate (150 kg ha^{-1}) resulted in lower value of electrical conductivity.

Seedling growing

A cover crop × N rate interaction was detected for dry seedling biomass after germination and after accelerated aging (Fig 2). Common bean plants under a single-specie cover crop (pear millet - *P. glaucum*; PM) produced seeds with a lower seedling dry biomass after germination compared to all cover crop mixtures when 50 kg N ha^{-1} was applied (Fig 2a). Common beans plants under PM-PP residues produced seeds with higher seedling dry biomass after germination values in comparison to PM and PM-JB under the highest N fertilization rate (150 kg ha^{-1}). Within the PM-VB treatment, the application of 50 kg N ha^{-1} resulted in seedlings with a higher dry biomass than the control.

For the seedling dry biomass after accelerated aging, higher values were observed for common bean seeds produced under PM-SH than PM, PM-JB, and PM-VB when no N fertilizer was added (Fig 2b). Common bean plants under PM-PP produced seeds with higher seedling dry biomass compared to PM-JB and PM-VB at 50 kg ha^{-1}. At 150 kg N ha^{-1}, common bean seeds produced PM-PP exhibited a higher seedling dry biomass than PM-JB. Across cover crop combinations, 50 kg N ha^{-1} resulted in higher seedling dry biomass values than the control for PM-PP. Additionally, for PM-SH, the control and 50 kg N ha^{-1} treatments increased common bean seedling dry biomass compared to the 150 kg N ha^{-1} treatment.

Common bean seed N and protein concentrations were affected by the N fertilization rates (Fig 3ab). The common bean seeds obtained from the application of 100 kg N ha^{-1} showed higher N and protein concentrations compared to all other treatments.

The Pearson's correlation coefficient values are presented in Table 2. The first germination count, final germination, germination speed index, accelerated aging, N concentration, and protein concentration positively correlated with each other, with r varying from 0.29 to 0.94 (p<0.01). Electrical conductivity was negatively correlated with final germination, N concentration, and protein concentration (r ranging from -0.24 to -0.32; p<0.05). Seed N concentration was strongly correlated with seed protein content (r = 0.99; p<0.01).

Discussion

The consistent high value of first germination count for all treatments (cover crops x N rates) indicates that common bean seeds presented great vigor and uniformity. Seeds with high vigor have a superior initial performance due to a faster germination compared to those with low vigor, which usually requires more time to germinate (Marcos Filho, 2005). This is likely related to physiological (composition and integrity of cell membranes) and sanitary (incidence of diseases) problems. Similarly, common bean seeds presented final germination rates >90%, which is higher than the minimum (80%) required for production and marketing of common bean seeds in Brazil (MAPA, 2013).

Table 1. Analysis of variance (ANOVA) for cover crop and fertilizer N rate and their interaction on common bean parameters[a].

Factor	FGC	FG	AA	GSI	EC	SDBG	SDBAA	NC	PC
Cover crop	<0.001**	0.019*	0.001**	0.020*	<0.001**	<0.001**	<0.001**	0.280	0.427
N rate	<0.001**	<0.001**	0.001**	<0.001**	<0.001**	0.423	0.001**	<0.001**	<0.001**
Cover crop × N rate	0.421	0.630	0.755	0.080	0.196	0.010*	0.017*	0.362	0.513

[a] FGC: first germination count; FG: final germination; AA: accelerated aging; GSI: germination speed index; EC: electrical conductivity; SDBG: seedling dry biomass after germination; SDBAA: seedling dry biomass after accelerated aging; NC: N concentration in seed; PC: protein concentration in seed.

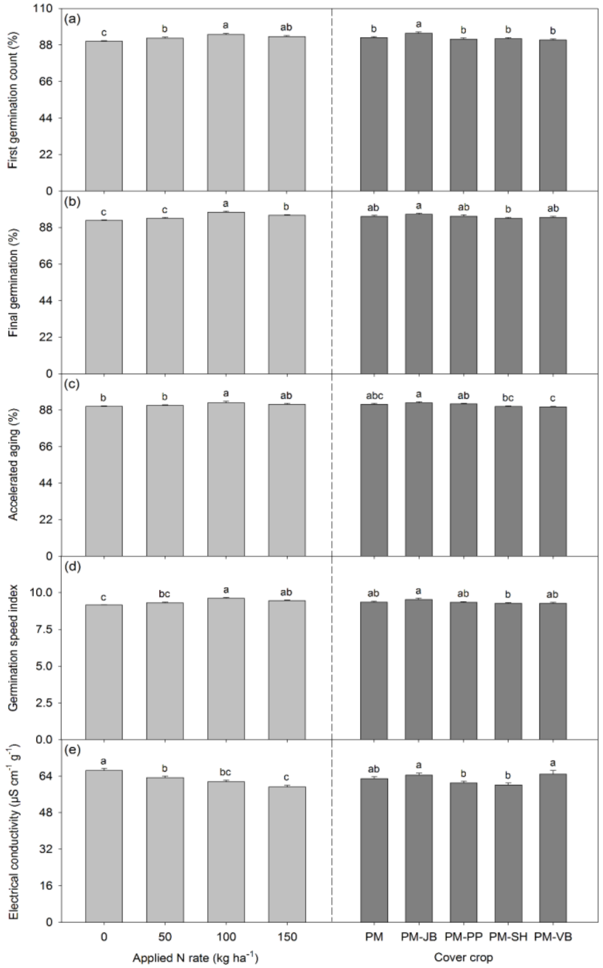

Fig 1. Main effect of fertilizer N rate and cover crop residues influencing the following physiological quality parameters of common bean seed: first germination count (a), final germination (b), accelerated aging (c), germination speed index (d), and electrical conductivity (e). PM: pear millet; PM-JB: pear millet-jack bean; PM-PP: pear millet-pigeon pea; PM-SH: pear millet-sunn hemp; and PM-VB: pear millet-velvet bean. The error bars indicate the SEM (*n*=20 and 16 for applied N rate and cover crop, respectively). Letters indicate differences between treatments according to Tukey's HSD test ($p<0.05$).

Table 2. Pearson's correlation coefficients between common bean response parameters[a] as influenced by cover crop and N fertilization.

Variable	FGC	FG	AA	GSI	EC	SDBG	SDBAA	NC	PC
FCG	1.00								
FG	0.64**	1.00							
AA	0.39**	0.56**	1.00						
GSI	0.86**	0.94**	0.55**	1.00					
EC	-0.12	-0.24*	-0.12	-0.21	1.00				
SDBG	-0.07	0.05	0.01	0.01	-0.17	1.00			
SDBAA	-0.01	0.02	0.03	0.01	-0.28*	0.13	1.00		
NC	0.31**	0.43**	0.29**	0.42**	-0.31**	-0.01	0.07	1.00	
PC	0.31**	0.42**	0.30**	0.42**	-0.32**	-0.01	0.07	0.99**	1.00

[a] FGC: first germination count; FG: final germination; AA: accelerated aging; GSI: germination speed index; EC: electrical conductivity; SDBG: seedling dry biomass after germination; SDBAA: seedling dry biomass after accelerated aging; NC: N content in seed; PC: protein concentration in seed.
*: p <0.05; **: p <0.01.

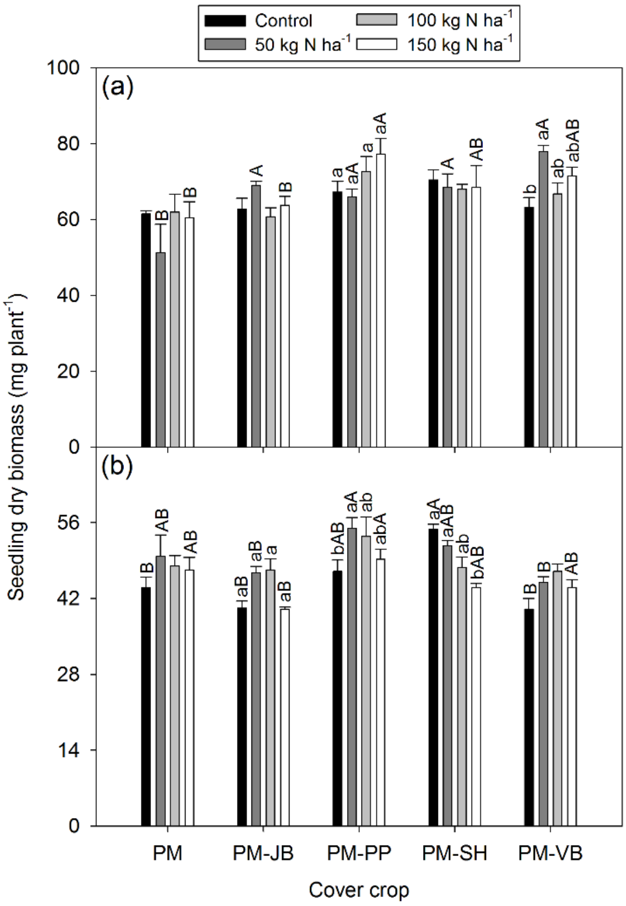

Fig 2. Seedling dry biomass after germination (a) and accelerated aging (b) as influenced by cover crop and fertilizer N rate. PM: pear millet; PM-JB: pear millet-jack bean; PM-PP: pear millet-pigeon pea; PM-SH: pear millet-sunn hemp; and PM-VB: pear millet-velvet bean. The error bars indicate SEM (n=4). Lowercase letters indicate differences between N rates within respective cover crops, while capital letters indicate differences between cover crops treatments within respective N rates, both according to Tukey's HSD test (p<0.05).

Although differences were found among treatments, the magnitude of difference in final germination between common seeds produced under PM-JB (which had the highest final germination value; 96%) and PM-SH (which had the lowest germination value; 94%) was extremely low (2%). Furthermore, as no difference was found between common bean seeds produced under single-specie (PM) and mixed cover crops (with the exception of PM-SH), this suggests that pear millet can be used as a monoculture or in a consortium with legumes. The importance of cover crops in protecting the soil surface and therefore increasing plant-available water is well-known (Nielsen et al., 2015) and may have a key role in seed production. Abrantes et al. (2015) and Silva et al. (2017) reported that common beans plants under straw-covered soils produced seeds with higher final germination percentage (92-98%) compared to bare soils (76-88%). Furthermore, the use of cover crops may lead to an increase of nutrient availability to the parent plant during the period of seed filling, resulting in high quality seeds. As related to the N fertilizer effect, similar results were obtained in Farinelli et al. (2006), which reported a higher final germination of common bean seeds as a result of high

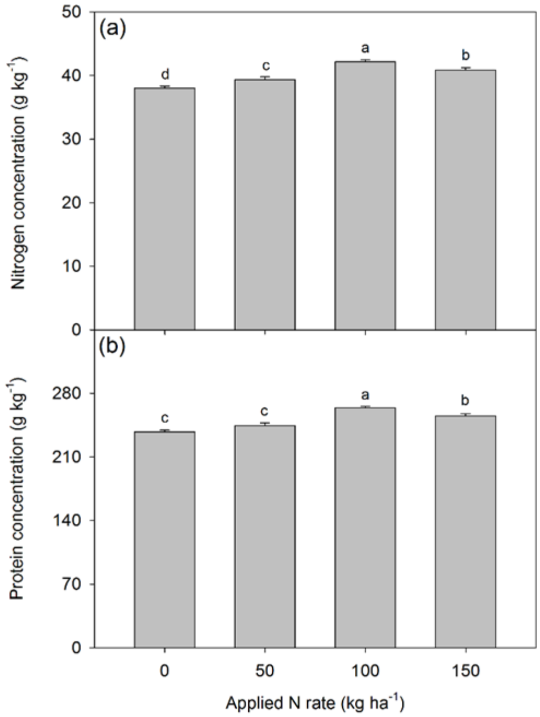

Fig 3. Main effect of fertilizer N rate influencing N (a) and protein concentration (b) in common bean seed. The error bars indicate the SEM (n=16). Letters indicate differences between treatments according to Tukey's HSD test (p<0.05).

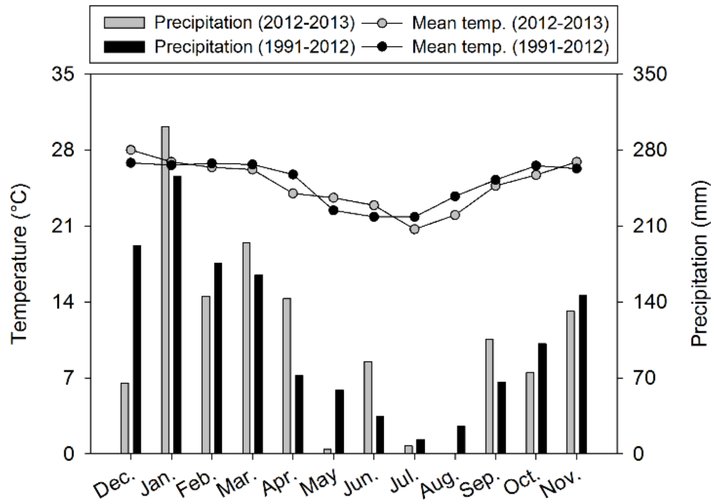

Fig 4. Monthly mean air temperature and precipitation of the 2012-2013 growing season and monthly long-term average (period 1991-2012).

N input. Hampton (2002) reported that proper availability of nutrients (mainly N and P), and water during reproductive stages could increase final germination percentage and subsequently the seedling vigor. However, our results contrast to those obtained by Crusciol et al. (2003) and Goes et al. (2011), which did not find a difference in the final germination of two Brazilian cultivars of common bean resulting from N fertilization. Germination may vary according to the cultivar, nutritional status, disease and pest damage, and management practices. Thus, it is possible that some common bean cultivars interact more favorably with the environment than others (Michels et al., 2014). The strong positive correlation between the final germination and germination speed index (r = 0.94) explain the same pattern verified for both attributes. Seeds with lower vigor have a lower water uptake rate (imbibition), which consequently delay its germination and seedling development (Woodstock, 1988). The degree of permeability and hardness of the testa (seed coat) plays a fundamental role in the imbibition period (Woodstock, 1988). For the accelerated aging test, we observed a reduction in common bean seeds germination after being exposed to high temperature and relative humidity. The accelerated aging test is one of the most sensitive and efficient vigor test (Marcos Filho, 2005). According to Marcos Filho (1999) the stressful conditions that seeds are submitted may increase respiration and promote a drastic reduction in germination. In general, all treatments (cover crop x N rates) presented a reduction between 3 to 5% compared to the final germination (which offers the perfect temperature and humidity conditions for seed germination). Therefore, even under extreme conditions, common bean seeds presented high quality and vigor. Regardless cover crop usage, similar results were obtained by Farinelli et al. (2006), which reported an increase in seed vigor when high N rates were used. In contrast, Ambrosano et al. (1999) and Carvalho et al. (2001) did not observe differences in germination rates after accelerated aging when N fertilizer was applied to common bean.

The vigor of common bean seeds was also influenced by cover crops and N fertilization when evaluated by the electrical conductivity test. However, considering the main effect of cover crops, low variations in the quantity of electrolytes leakage from common bean seeds was observed among the treatments (maximum difference of 3.9 μS cm^{-1} g^{-1}). To our knowledge, there are no reports evaluating the effect of cover crops on electrical conductivity of common bean seeds, preventing us from making comparisons. Furthermore, we observed a reduction in electrical conductivity values with the increase of the N rate. In contrast, Crusciol et al. (2003) and Farinelli et al. (2006) did not observe any differences in the leakage of electrolytes from common bean seeds. Environmental factors (e.g., drought, nutritional status, disease and pest damage) may affect seed formation process, primarily the integrity of cell membranes. Seeds produced under stress conditions can have thinner cell membranes and a higher quantity of soluble ions leached during the imbibition process (Vieira et al., 2002). Seeds with lower vigor have a greater release of exudates in the solution and higher intensity of disorganization of the cell membrane systems (Carvalho et al., 2009; Silva et al., 2014). Our results are similar to those previously reported by Levitt (1980), who also indicated a negative correlation between seed electrical conductivity and germination (here defined as final germination).

A complex interaction between cover crop and N rate was observed for common bean seedling dry biomass after germination and accelerating aging tests. In general, cover crop mixtures and high N fertilization rates resulted in higher seedling dry biomass after germination. We believe that the lower C/N ratio of the cover crop mixtures and the addition of N fertilizer increased N availability in the soil to the parent plant during the period of seed filling, resulting in more vigorous seeds (Weil and Brady, 2016). The same trend was observed for seedling dry biomass after the accelerated aging test, although a reduction in dry weight was observed after common bean seeds were exposed to high temperature and relative humidity. According to Hampton and Tekrony (1995) the seedling dry biomass is an important index of seedling vigor and is used as a criterion for vigor assessment.

Nitrogen fertilization improved N and protein concentration in common bean seeds, regardless of the cover crop used. Common bean plants fertilized with 100 N kg ha^{-1} produced seeds with in the highest N concentration, and therefore, higher protein compared to the other treatments. Similar results were observed by Gomes Junior and Sá (2010), who found that the application of 90 kg N ha^{-1} resulted in greater common bean seed N and protein concentrations. Nitrogen is an important nutrient for seed filling, and N fertilization may consequently increase protein concentration, which is a good index for seed quality and vigor. Conversely, Arf et al. (1999) and Farinelli et al. (2006) did not observe an increase of N and protein concentration in common bean seeds after N fertilization. Finally, we believe that N fertilization may have lowered the C/N ratio of the cover crop residues, resulting in net mineralization, and consequently, increased N availability in the soil (Weil and Brady, 2016). Thus, adequate soil N availability throughout the experiment improved the production of high quality seeds, which posse high crude and soluble protein content, as well as non-proteic compounds such as amino acids and peptides (Gomes Junior and Sá, 2010).

Materials and methods

Site description

The experiment was carried out in Selvíria (20°22'S, 51°24'W, 340 m a.s.l.), Mato Grosso do Sul, Brazil, from Dec. 2012 to Jul. 2013. The no-till system was established in the experimental area in 2009. The following crops are usually grown in a rotation system: soybean [*Glycine ma*x. (L.) Merr.], maize (*Zea mays* L.), and common bean. The soil at the study site is classified as Typic Acrustox with a sandy clay loam texture (Soil Survey Staff, 2014). Before the start of the experiment, individual soil samples (*n*=4) were randomly collected at the 0-0.20 m depth, combined to obtain a composite sample, and submitted for physicochemical characterization according to Raij (2001). The following soil properties were detected at the superficial layer: pH (0.01 M $CaCl_2$) 4.9; organic C (Walkley-Black) 13 g dm^{-3}; P (anionic-exchange resin) 36 mg dm^{-3}; K, Ca, and Mg (cationic-exchange resin) 5.2, 25, and 19 $mmol_c$ dm^{-3}, respectively; potential acidity (H+Al; SMP pH buffer) 36 $mmol_c$ dm^{-3}; cation exchange capacity at pH 7.0 (CEC; summation of K, Ca, Mg, and H+Al) 85 $mmol_c$ dm^{-3}; and base saturation (BS; summation of K, Ca, and Mg divided by CEC at pH 7.0, multiplied by 100) 58%. The precipitation and temperature recorded during the growing season and long-term average (21-yr average) are shown in Fig 4. The annual mean temperature and precipitation of the 2012/2013 growing season was 24.8°C and 1258 mm, respectively, which was very similar to the long-term average (25.0°C, 1306 mm).

Experimental setup

A 5 × 4 factorial experiment arranged in a randomized complete block design was performed, with four replicates each. The treatments included cover crops and N fertilizer rates. Cover crop treatments comprised a single-grass specie (pear millet; *Pennisetum glaucum*; PM) or grass-legume mixtures as follows: pear millet-jack bean (*P. glaucum*; *Canavalia ensiformis*; PM-JB), pear millet-pigeon pea (*P. glaucum*; *Cajanus cajan*; PM-PP), pear millet-sunn hemp (*P. glaucum*; *Crotalaria juncea*; PM-SH), and pear millet-velvet bean (*P. glaucum*; *Mucuna pruriens*; PM-VB). The second factor, N fertilizer rates (50, 100, and 150 kg N ha^{-1}) were topdressed applied to common bean. A control (unfertilized treatment) was also included.

Cover crops were planted manually on Dec. 11, 2012, in succession to maize (*Zea mays* L.), without fertilizer amendment. Each plot consisted of eight rows of 20 m length with 0.5 m row spacing. The seed density for each of the cover crops was 80, 12, 20, 30, 10 seeds m^{-1} of pear millet, jack bean, pigeonpea, sunn hemp, and velvet bean, respectively. At 82 d after emergence (DAE), the cover crops were crushed and flattened using a knife roller, followed by desiccation (1560 g ha^{-1} glyphosate) at 85 DAE. The crop residues were left on the soil surface and used as straw mulch for common bean, the cash crop in rotation. On May 20, 2013, the common bean was mechanically planted using a seed-cum-fertilizer drill. The cultivar used was the IAC Formoso (carioca commercial group and indeterminate growth habit) at a density of 15 seeds m^{-1}, aiming to reach 240,000 plants ha^{-1}. The plots were formed by 6 rows with 5 m length each, spaced at 0.50 m. Seeds were chemically treated with carboxin-thiram fungicide at a rate of 2 mL kg^{-1} of seeds. Fertilization at seeding time was performed by applying 10, 33, and 21 kg of N (as urea), P (as triple superphosphate), and K (as potassium chloride), respectively, based on recommendations of Ambrosano et al. (1997). Seedling emergence occurred 6 d after seeding. Nitrogen in topdressing fertilization was performed at 24 DAE, at the V4 stage (third trifoliate leaf), at a rate of 50, 100, 150 kg N ha^{-1}, as urea. Fertilizer N was surface hand-applied in single-side banding (8 cm width), ~10 cm from the crop row, in single application. To reduce ammonia losses by volatilization from urea application, the center-pivot sprinkler system irrigated the experimental site (application rate of 5-6 mm) to incorporate the fertilizer into the soil. At maturity (66 DAE), common bean plants within the two central rows (2 rows × 4 m long; 4 m^2 area) in each plot were hand-harvested. Subsequently, plants were mechanically threshed and cleaned. Seeds were air-dried to a reach a moisture content of 13% (wet basis) and stored in gas-permeable paper bags at 20°C and relative humidity ~60% until analysis.

Seed quality measurements

The germination test was performed using a completely randomized designed with four replicates of 50 seeds of each treatment. Germitest paper was moistened with distilled water at a volume equal to 2.5 times the dry weight. Germitest paper rolls were then placed into a germinator set at 25°C. The first and final counting were performed at 5 and 9 d after incubation (DAI) respectively, and the results were expressed in normal seedlings percentage (Brasil, 2009). The first germination count was performed together with the germination test to estimate the percentage of normal seedlings (Brasil, 2009). The germination speed index was also evaluated according to Maguire (1962). Thus, the first counting and germination speed index represented the seed vigor, while the final count its viability. Dry seedling biomass was determined at the first germination count (Nakagawa, 1999; Brasil, 2009). Four subsamples composed of 10 normal seedlings, discarding the cotyledons, were randomly taken from each treatment. Subsequently, seedlings were oven-dried at 80°C until a constant weight. The total biomass of each subsample was divided by 10 (number of normal seedlings used), resulting in the average dry biomass per seedling.

Accelerated aging was performed using 200 seeds per treatment. Seeds were uniformly spread over a stainless-steel mesh fixed inside plastic boxes of gerbox type (11.0 × 11.0 × 3.0 cm), containing 40 mL of distilled water at the bottom. The boxes were closed and placed into a BOD incubator (model SL-224, Solab, Piracicaba, Brazil) at 42°C for 60 h (Marcos Filho, 1999). Subsequently, four subsamples of 50 seeds were evaluated through the germination test as described previously (Brasil, 2009). Therefore, the counting of normal seedlings was carried out at 5 DAI. In addition, the dry seedling biomass after accelerated aging was measured as for the germination test. Electrical conductivity was measured using four replications of 50 seeds each. The seeds were weighed, placed in plastic cups containing 75 mL of distilled water and taken to the aforementioned BOD incubator at 25°C for 24 h. After this period, the electrical conductivity measurement was obtained by immersing a glass electrode into the solution (Vieira and Krzyzanowski, 1999). The seed N content was determined using four replicates of 50 seeds of each treatment. Briefly, seeds were oven-dried at 65ºC to a constant weight, finely ground, passed through a 0.50-mm sieve, and analyzed for total N concentration by digestion with H_2SO_4 and H_2O_2 followed by steam distillation and titration with 0.025 M H_2SO_4 (Malavolta et al., 1997). Seed protein concentration was calculated by multiplying the total N content by a correction factor of 6.25 (AOAC, 2006).

Statistical analysis

The results obtained from different treatments were subjected to a two-way ANOVA using the GLM procedure of SAS (version 9.3, SAS Institute Inc.). Cover crops and N fertilization were considered as fixed factors within the statistical model. The *F*-test was used followed by the Tukey's honest significant difference (HSD) test to separate means ($p \leq 0.05$). The relationships between variables were evaluated by Pearson's correlations using the CORR procedure. All graphs were generated using SigmaPlot (version 11.0, Systat Software Inc.).

Conclusion

Based on our results, single-specie and mixed cover crop treatments variably influence (although with low intensity) the physiological quality of common bean seeds. Therefore, the cover crop treatments tested can be used interchangeably before common bean grown. Overall, the application of 100 kg N ha^{-1} as topdressing slightly increase the vigor and protein content of common bean seeds compared to the other N rate treatments. Although the combination of cover crops and N fertilization under no-till farming had limited effect on common bean seed quality in this study, the proper N fertilization management coupled with no-till farming with crop residues over the soil surface is a well-known avenue to improve common been seed yield.

Acknowledgments

We thank Jonathan Lin (University of California, Davis) for critical comments and review of the written English in the manuscript. The first author (AP) was supported by a scholarship from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grant #2012/10985-1). This work was funded by the Conselho Nacional de

Desenvolvimento Científico e Tecnológico (CNPq; grant #309842/2010-7).

References

Abrantes FL, Sá ME, Silva MP, Souza LCD, Arruda N (2015) Qualidade fisiológica de sementes de feijão em função de sistemas de manejo de solo e adubação foliar com boro. Cult Agron. 24:167-180.

Amaral CB, Pinto CC, Araújo Flôres J, Mingotte FLC, Lemos LB, Fornasieri Filho D (2016) Produtividade e qualidade do feijoeiro cultivado sobre palhadas de gramíneas e adubado com nitrogênio em plantio direto. Pesq Agropecu Bras. 51:1602-1609.

Ambrosano EJ, Tanaka RT, Mascarenhas HAA, Raij BV, Quaggio JA, Cantarella H (1997) Leguminosas e oleaginosas. In: Raij BV, Cantarella H, Quaggio JA, Furlani AMC (eds) Recomendações de adubação e calagem para o estado de São Paulo, 2nd edn. IAC, Campinas.

Ambrosano EJ, Ambrosano GMB, Wutke EB, Bulisani EA, Martins ALM, Silveira LCP (1999) Efeitos da adubação nitrogenada e com micronutrientes na qualidade de sementes do feijoeiro cultivar IAC-Carioca. Bragantia. 58:393-399.

Aoac International (2006) Official methods of analysis of AOAC International, 19th edn. AOAC International, Arlington.

Arf O, Silva LS, Buzetti S, Alves MC, Sá ME, Rodrigues RAF, Hernandez FBT (1999) Efeito da rotação de culturas, adubação verde e nitrogenada sobre o rendimento do feijão. Pesq Agropecu Bras. 34:2029-2036.

Brasil (2009) Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento, 1st edn. Mapa, Brasília. 399.

Broughton WJ, Hernández G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.) - model food legumes. Plant Soil. 252:55-128.

Carvalho MAC, Arf O, Sá ME, Buzetti S, Santos NCB, Bassan DAZ (2001) Produtividade e qualidade de sementes de feijoeiro (*Phaseolus vulgaris* L.) sob influência de parcelamentos e fontes de nitrogênio. Rev Bras Cienc Solo. 25:617-624.

Carvalho LF, Sediyama CS, Reis MS, Dias DCFS, Moreira MA (2009) Influência da temperatura de embebição da semente de soja no teste de condutividade elétrica para avaliação da qualidade fisiológica. Rev Bras Sementes. 31:9-17.

Coelho FC, Vieira C, Mosquim PR, Cassini STA (1998) Nitrogênio e molibdénio nas culturas do milho e do feijão, em monocultivos e em consórcio: I Efeitos sobre o feijão. Rev Ceres. 45:393-407.

Conab - Companhia Nacional de Abastecimento (2018) Acompanhamento da safra brasileira de grãos: Décimo segundo levantamento da safra 2016/17. Available at: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/17_09_12_10_14_36_ boletim_graos_setembro_2017.pdf. (Accessed 24 Feb. 2018).

Crusciol CAC, Lima ED, Andreotti M, Nakagawa J, Lemos LB, Marubayashi OM (2003) Efeito do nitrogênio sobre a qualidade fisiológica, produtividade e características de sementes de feijão. Rev Bras Sementes. 25:108-115.

Decker AM, Clark AJ, Meisinger JJ, Mulford FR, McIntosh MS (1994) Legume cover crop contributions to no-tillage corn production. Agron J. 86:126-135.

Fageria NK, Baligar VC, Bailey BA (2005) Role of cover crops in improving soil and row crop productivity. Commun Soil Sci Plan. 36:2733-2757.

Farinelli R, Lemos LB, Cavariani C, Nakagawa J (2006) Produtividade e qualidade fisiológica de sementes de feijão em função de sistemas de manejo de solo e adubação nitrogenada. Rev Bras Sementes. 28:102-109.

França Neto JB, Krzyzanowski FC, Henning AA (2010) A importância do uso de semente de soja de alta qualidade. Infor Abrates. 20:37-38.

Goes RJ, Lopes AS, Filho SNS, Oliveira GQ (2011) Qualidade fisiológica de sementes de feijão sob manejos de irrigação e doses de nitrogênio. Rev FZVA. 18:155-164.

Gomes Junior FG, Sá ME (2010) Proteína e qualidade de sementes de feijão (*Phaseolus vulgaris* L.) em função da adubação nitrogenada em plantio direto. Rev Bras Sementes. 32:34-44.

Hampton JG, Tekrony BH (1995) Conductivity test. In Hampton JG, Tekrony BM (eds) Handbook of vigor methods, 3rd ed. ISTA, Zürich.

Hampton JG (2002) What is seed quality? Seed Sci Technol. 30:1-10.

Levitt J (1980) Responses of plant to environmental stresses, 1st edn. Academic Press, New York.

Maguire JD (1962) Speed of germination-aid in selection and evaluation for seeding emergence and vigor. Crop Sci. 2:176-177.

Malavolta E, Vitti GC, Oliveira SA (1997) Avaliação do estado nutricional das plantas: princípios e aplicações, 1st edn. Potafos, Piracicaba.

Marcos Filho J (1999) Teste de envelhecimento acelerado. In: Krzyzanowski FC, Vieira RD, França Neto JB (eds) Vigor de sementes: conceitos e testes. Abrates, Londrina.

Marcos Filho J (2005) Fisiologia de sementes de plantas cultivadas. Fealq, Piracicaba.

Mapa - Ministério da Agricultura, Pecuária e Abastecimento (2013) Instrução Normativa, n° 45, de 17 de setembro de 2013. Estabelece normas especificas e padrões de identidade e qualidade para a produção e comercialização de sementes de grandes culturas. Diário Oficial da União: Brasília, DF, de 18 de setembro de 2013. Seção 1, p. 16. Available at: http://www.agricultura.pr.gov.br/arquivos/File/PDF/padroes_feijao.pdf. (Accessed 24 Feb 2018).

Michels AF, Souza CA, Coelho CMM, Zilio M (2014) Qualidade fisiológica de sementes de feijão crioulo produzidas no oeste e planalto catarinense. Rev Cienc Agron. 45:620-632.

Nakagawa J (1999) Testes de vigor baseados no desempenho das plântulas. In: Krzyzanowski FC, Vieira RD, França Neto JB (eds) Vigor de sementes: conceitos e testes. Abrates, Londrina.

Nielsen DC, Lyon DJ, Hergert GW, Higgins RK, Holman JD (2015) Cover crop biomass production and water use in the Central Great Plains. Agron J. 107:2047-2058.

Raij BV, Quaggio JA, Cantarella H, Abreu CA (2001) Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. In: Raij BV, Andrade JC, Cantarella H (eds) Análise química para avaliação da fertilidade de solos tropicais, 2nd edn. IAC, Campinas.

Silva MG, Arf O, Sá ME, Rodrigues RAF, Buzetti S (2004) Nitrogen fertilization and soil management of winter common bean crop. Sci Agric. 61:307-312.

Silva VN, Zambiasi CA, Tilmann MAA, Menezes NL, Villela FA (2014) Condução do teste de condutividade elétrica utilizando partes de sementes de feijão. Rev Cienc Agrar. 37:206-213.

Silva MP, Arf O, Sá ME, Abrantes FL, Berti CLF (2017) Influência da adubação fosfatada e coberturas vegetais na qualidade de sementes de feijão. Rev Agrotec. 8:39-45.

Soil Survey Staff (2014) Keys to soil taxonomy, 12th edn. USDA-Natural Resources Conservation Service, Washington.

Teasdale JR (1996) Contribution of Cover Crops to Weed Management in Sustainable Agricultural Systems. J Prod Agric. 9:475-479.

Vieira RD, Krzyzanowski FC (1999) Teste de condutividade elétrica. In: Krzyzanowski FC, Vieira RD, França Neto JB (eds) Vigor de sementes: conceitos e testes. Abrates, Londrina.

Vieira RD, Penariol AL, Perecin D, Panobianco M (2002) Condutividade elétrica e teor de água inicial das sementes de soja. Pesq Agropecu Bras. 37:1333-1338.

Zucareli C, Brzezinski CR, Werner F, Abati J, Nakagawa J (2016) Physiological quality of the seeds of common bean cultivars grown in different phosphorus levels and growing seasons. Semin Cienc Agrar. 37:3859-3870.

Weil RR, Brady NC (2016) The nature and properties of soils, 11th edn. Pearson, London.

Woodstock LW (1988) Seed imbibition: a critical period for successful germination. J Seed Technol. 12:1-15.