Original Article

BIOCHEMICAL BEHAVIOR OF BEAN SEEDS AND GRAINS DURING STORAGE

COMPORTAMENTO BIOQUÍMICO DE SEMENTES E GRÃOS DE FEIJÃO DURANTE O ARMAZENAMENTO

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ABSTRACT: The development of researches about vigor provides greater insight about the physiological behavior of seeds over storage periods. The aim of this study was to evaluate the biochemical changes which occurred in beans stored for 360 days. Analyses were performed at 0, 30, 90, 180, 270, and 360 days after receiving the seeds (S1 and S2) and grains (G1 and G2) of BRS Splendor. The following tests were conducted: water content, electrical conductivity, and chemical composition of the soaking solution, where sugars and ions Fe^{2+}, Zn, Ca^{2+}, Mg^{2+} and K^{+} were quantified. The experimental design was completely randomized split-plot and means were compared through Tukey test (p>0.05). Water content decreased after 90 days, and after 180 days reached an average of 16%. The electrical conductivity showed the greatest loss of solute after 90 days. Larger amounts of soluble sugars were found in S1 and G1. The quantities of potassium and calcium decreased after 360 days, while magnesium increased after 90 days of storage. The electrical conductivity test showed no direct relation to the quantification of the investigated ions. The amount and behavior of amino acids and studied ions did not showed relation to the decrease in vigor of beans.

PALAVRAS-CHAVE: *Phaseolus vulgaris* L. Vigor. Electrical conductivity. Electrolytes leaching.

INTRODUCTION

The global increase in food demand establishes a need to make better use of cultivable lands. For this purpose, it is necessary to use certified seeds, in order to increase crop productivity. Seeds such as bean *(Phaseolus vulgaris* L.), one of the most consumed grains in Brazil, that when combined with rice provides a balanced diet, with proteins, carbohydrates, minerals, fibres, vitamins and amino acids (RIGUEIRA et al., 2009).

Quality control programs seek to evaluate seed physiological potential, in order to set a direct relationship between seed vigor and crop establishment, key attributes to agricultural development (COIMBRA et al., 2009). To that extent, usage of tests that are sensitive to biochemical alterations, during seed aging, becomes very relevant to estimate viability and storage potential (COSTA et al., 2007).

The beginning of the seed deterioration process can be indicated by the destruction of cell membranes system (SANTOS et al., 2005; COSTA et al., 2007). And it can be quantified through electric conductivity test. Vigorous seeds present a greater repairing capacity of damages in cell membranes. Therefore, during soaking, the electrolytic leaching is smaller (MARCOS FILHO, 2005; BINOTTI et al., 2008; COSTA et al., 2008).

The leached of the electrical conductivity test is composed, mainly, of sugars, amino acids and inorganic ions, such as potassium, calcium, magnesium, sodium and manganese. High concentrations - of the above substances - can be an indicative of vigor and germination loss (MARCOS FILHO, 2005; BINOTTI et al., 2008). Among the best studied electrolytes, to try establishing a correlation with physiological quality of seeds, are calcium, magnesium and potassium, which are researched by many authors (BINOTTI et al., 2008; COSTA et al., 2008; FESSEL et al., 2010; SILVA et al., 2012).

In face of the need for more information regarding fast and objective tests of vigor, and its relationship with seed longevity; this work aimed to evaluate the biochemical behavior of bean seeds and grains, and its relation to the physiological quality and longevity of beans during 360 days of storage, under natural conditions.

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MATERIAL AND METHODS

The tests were conducted using BRS Splendor beans (*P. vulgaris*, black group), from the second harvest (dry) of 2011, cultivated in two farms of Cascavel city, field 1 (24° 53’ 49.9’’ S and 53° 31’ 36.1’’ W) and field 2 (24° 49’ 36.0’’ S and 53° 32’ 29.1’’ W), in western Paraná.

The fields were being used for seeds reproduction and only three meters around the area are marketed for consumption (grain), as established by Agriculture Ministry (BRASIL, 2011). Thereby, beans were classified as: field seeds 1 (S1), field seeds 2 (S2), field grains 1 (G1) and field grains 2 (G2).

Samples were separated and stored into paper Kraft bags, under non-controlled environmental conditions, for 360 days. Evaluations to determine biochemical quality were performed when samples arrived, and after 30; 90; 180; 270 and 360 days of storage.

Water content was determined through standard oven method, at 105 ±3 ºC, for 24 hours, in accordance with methods indicated by Rules for Seeds Analyze - RAS (BRASIL, 2009).

Samples containing 50 beans were weighted and placed in a recipient containing 75 mL of deionized water, and kept at 25 ºC for 24 hours, afterwards, electrical conductivity reading was performed (VIEIRA; KRZYZANOWSKI, 1999).

Soluble sugar quantification was performed following Dubois (1956), 0.5 mL of aqueous solution of 5% phenol and 2.5 mL of concentrated sulphuric acid were added to 0.5 mL of the soaking water. The prepared was agitated and left to rest for 15 minutes. With samples colder, reading was performed in spectrophotometer at 490 nm. The results were compared with glucose standard curve.

Iron (Fe^{2+}), Zinc (Zn), Calcium (Ca^{2+}), Magnesium (Mg^{2+}) and Potassium (K^{+}) ions were determined from the soaking water, through direct reading in flame atomic absorption spectrophotometer (BINOTTI et al., 2008).

A completely randomized split-plot design was used (CRD), containing four replications. The results were submitted to analysis of variance, average comparison test and regression analysis through Tukey (p>0,05), software R, version 2.15.1 (R DEVELOPMENT CORE TEAM, 2011).

RESULTS AND DISCUSSION

A decrease in water content was observed after 90 days of storage. Hydration of beans was noticed after 180 days of storage, resulting in average values of 16% on water content, by the end of the experiment. In this study, oscillation of water content in relation to relative humidity modifications of storage period was observed, showing this water vapor exchange capacity to reach hygroscopic balance. These variations may be responsible for the behavior of the variables described below.

Table 1. Electrical conductivity of beans, from Splendor BRS variety, during storage periods.

Beans	Electrical conductivy (µS.cm^{-1}g^{-1})					
	Storage periods (days)					
	0	30	90	180	270	360
S1	104.72aAB	98.19aCD	113.28aA	91.69aD	110.34aBC	106.67aAB
S2	98.01bAB	82.13bCD	100.39bA	79.18bD	86.55bBC	91.70bAB
G1	118.06aAB	102.30aCD	118.23aA	92.68aD	101.45aBC	114.89aAB
G2	92.79bAB	81.96bCD	98.77bA	79.22bD	86.97bBC	103.31bAB

Means followed by case letter: comparison between columns and rows differ between them by Tukey test (5% of significance). S1 and G1 seeds and grains from field 1, S2 and G2 seeds and grains from field 2, respectively.

After 90 days of storage the biggest electrolytic leaching was observed, and after 180 days, the smaller one (Table 1). However, variation in electrical conductivity values, over the experiment, indicates that the test wasn’t sensitive enough to detect a decrease in beans vigor, corroborating with Silva et al. (2012), who studied beans of high vigor; with Fessel et al. (2010) who tested soy seeds stored at 10 and 20 ºC, and finally with Soares et al. (2010), using sorghum seeds.

However, the test was effective to distinguish beans vigor in Santos et al. (2005) work, and also to batches of beans studied by Cassol et al. (2012), during storage for eight and three months, respectively. The same test efficiency, to evaluate physiological quality, was proven by Coimbra et al. (2009) when testing sweet corn seeds, and by Braz and Rossetto (2009) regarding sunflower.

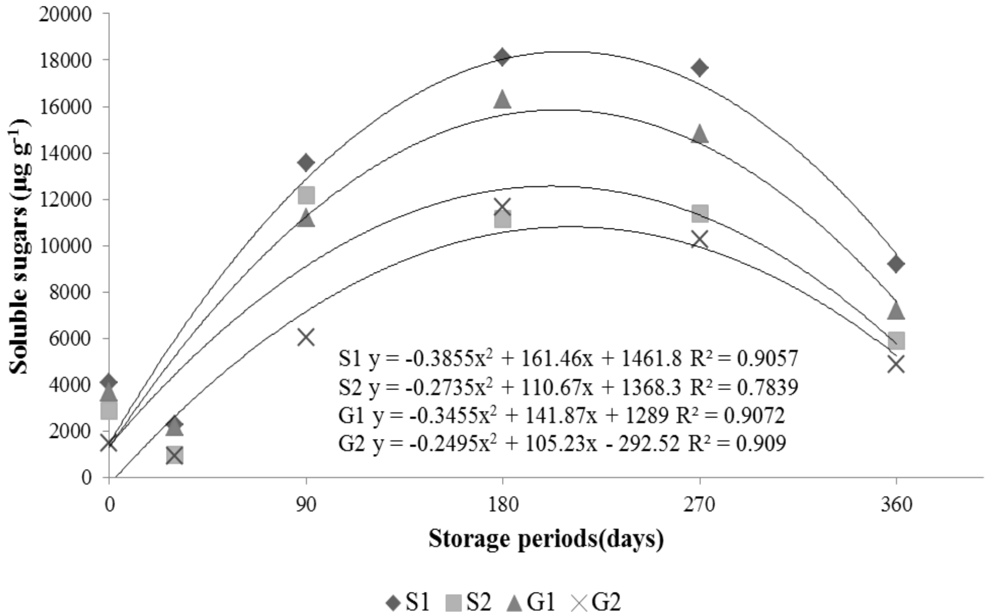

Figure 1. Significant regression model to soluble sugars content of beans, from Splendor BRS variety, during storage periods. S1 and G1 seeds and grains from field 1, S2 and G2 seeds and grains from field 2, respectively.

It can be seen, on figure 1, on average the biggest amount of soluble sugars is showed in beans of field 1, and the lowest averages are observed to G2. Lower values of this parameter were checked by Binotti et al. (2008), in their initial analysis, beans of Pearl cultivar. Being that, for this variable there was no significant interaction among factors. Similarly the beans, showed decrease after 30 days, following by increase after 180 days and a new drop of sugar levels.

These results differ from those obtained by Henning et al. (2010), which verified that vigorous seeds usually show great quantities of soluble sugars. In this matter, Marcos Filho (2005) states that during seeds deterioration, a decreasing of the sugars levels can be observed. The decline of this variable suggests the start of the deterioration process. This variation was inversely proportional to the water content, indicating an increase on basal metabolic rate. Water content increase may stimulate respiratory process, mobilization of reserves and energy release, stimulating the deterioration.

During the present study, there was some variation on iron content. For S1, the presence of Fe was not quantified at the last analysis. Obtained values remained between 0.000 and 1.029 to S1, 0.034 and 0.784 for S2, 0.357 and 0.798 for G1 and 0.062 and 0.889 µg g^{-1}, for G2. These results were lowers than the ones found by Pereira et al. (2011), while studying creole beans in two years of cultivation, which obtained Fe variation between 62 to 161.7 mg kg^{-1}. Also Mesquita et al. (2007) found greater quantities of this ion, 71.37 to 126.9 mg kg^{-1}, while investigating different beans lineages.

There was a reduction on zinc quantities (Table 2) until 270 days of storage, with a slight increase at the last analysis. Superior values were obtained by Pereira et al. (2011), between 32 a 68 mg kg^{-1}, when they evaluated two years of creole beans growth, while Binotti et al. (2008) didn't found any Fe or Zn in the electrical conductivity water of their experiment.

The beans presented similar behavior over the course of the research, regarding calcium quantities (Table 2). Values obtained in this work are lower than the ones obtained by Mesquita et al. (2007) (0.30 to 2.8 g kg^{-1}) when studying different beans lineages, and Pereira et al. (2011) (0.25 to 0.95 g kg^{-1}), featuring creole beans genotypes, whereas the present values are higher than the ones described by Binotti et al. (2008), 0.012 mg g^{-1}, at the first analysis of Pearl Beans.

According to Costa et al. (2008), calcium atomic radius is bigger than potassium and magnesium; this characteristic may have hampered calcium transposition through the membranes, in such way that they couldn't detect the presence of Ca in their trial using pea.

Once the interference of the atomic radius has been acknowledged, this may explain why Ca, Fe and Zn quantities were so low, since they have the biggest atomic radius between the studied ions of this work.

Table 2. Quantities of ions zinc, calcium and magnesium measured through the soaking water of beans, from Splendor BRS variety, during storage periods.

Beans	Zinc (μg g^{-1})					
	Storage periods (days)					
	0	30	90	180	270	360
S1	47.614aA	51.624bAB	49.350aB	39.581aB	23.478aB	29.250bB
S2	48.022abA	54.378bB	48.704aB	35.136aB	25.256aB	29.686bB
G1	39.244bBC	40.661aA	51.783aABC	32.642aBC	20.586aC	25.670aAB
G2	51.987aA	48.285abAB	48.296aBC	33.172aC	26.723aC	33.534aA
	Calcium (μg g^{-1})					
S1	8.711abAB	6.135abA	5.551aAB	5.316aBC	4.215aD	4.292abC
S2	7.959abA	5.001aA	5.962aA	3.682aB	3.194aC	3.530abB
G1	8.486bBC	7.902bAB	6.067aA	5.292aCD	4.396aE	6.751bDE
G2	8.238aA	6.327abA	4.794aA	3.657aBC	3.302aC	7.667aB
	Magnesium (μg g^{-1})					
S1	9.968aD	9.583aD	10.244aD	497.667bC	2571.638aB	4574.044aA
S2	9.143aD	8.828aD	9.404aD	491.462bC	2416.378aB	4414.543aA
G1	8.985aD	8.631aD	9.271aD	618.063aC	2203.513aB	4192.276aA
G2	9.280aD	8.789aD	9.445aD	616.374aC	2383.182aB	4372.079aA

Means followed by the same capital letter in the column and small letter in the line are not different according to (Tukey test, $p \geq 0.05\%$). S1 and G: seeds and grains from field 1; S2 and G2: seeds and grains from field 2.

Vanzolini & Nakagawa (2003) couldn't establish a connection between calcium leaching and physiological potential of peanut. Also, different lots of onions showed a decrease of calcium during a study carried out by Costa et al. (2007). Similar results were found by the present study, which wasn't able to use this variable (Ca) when analyzing seeds vigor. Whereas calcium increase was documented by Fessel et al. (2006) for corn, by Fessel et al. (2010) for soy seeds with high and low vigor, and by Silva et al. (2012) in lots of beans with lower physiological quality.

After 90 days of storage, a little amount of magnesium was found in the soaking water of beans (Table 2). Seeds and grains showed statistical differences after 180 days of storage, when grains released more magnesium than seeds. These values are higher than the ones obtained by Pereira et al. (2011), between 0.89 and 2.79 mg kg^{-1}, for creole beans.

These results disagree with the ones obtained by Costa et al. (2008), when testing pea seeds submitted to pre-hydration, it was possible to distinguish inferior lots using this parameter. Nevertheless, Vanzolini and Nakagawa (2003), analyzing the same test for peanut seeds were not able to distinguish amounts of Mg^{2+} of their lots, while Costa et al. (2007), studying the relationship between magnesium and physiological potential in onion seeds, noticed a downfall of this parameter during their work.

The present study discovered an increase of Mg^{2+} during storage periods, this result is also confirmed by Fessel et al. (2006), which tested corn seeds storage under higher temperatures, by Fessel et al. (2010) using soy seeds under the previous conditions and by Silva et al. (2012), which tested beans seeds of lower physiological quality. Alterations caused by deterioration modify the chemical composition of seeds, possibly causing magnesium increase, a less soluble ion (MARCOS FILHO, 2005).

Potassium quantities (Figure 2) showed a decrease after 90 days, followed by a discrete increase after 360 days of storage. But, according to Custódio and Marcos Filho *apud* Marcos Filho (2005), potassium release is directly related to the cell membranes permeability.

This greater permeability, as a rule, is related to a bigger deterioration. Kikuti et al. (2008) and Binotti et al. (2008) found a strong correlation between great release of K^+ and lower physiological quality of beans and peanut seeds. Different authors have been stated that potassium is the most released ion by seeds (VANZOLINI; NAKAGAWA, 2003; MARCOS FILHO, 2005; FESSEL et al., 2006; COSTA et al., 2008; FESSEL et al., 2010; SILVA et al., 2012). The results were higher than the ones observed by Binotti et al. (2008), for pearl beans (0.17 mg g^{-1}), and by Pereira et al. (2011), for creole beans in two years of cultivation (10.92 to 20.21 mg kg^{-1}). The increase of K+ concentration, in the water where seeds were immersed, is inversely related to the germination potential and seeds vigor, possibly being a rapid and indirect way to determine vigor, according to Binotti et al. (2008).

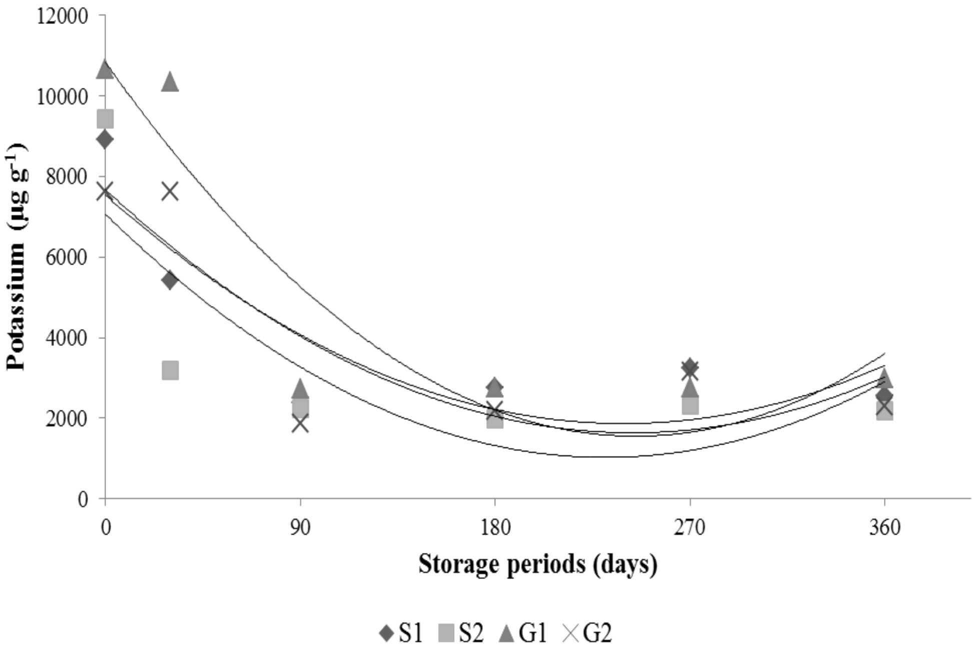

Figure 2. Potassium amount data of beans, from Splendor BRS variety, during storage periods. S1 and G1 seeds and grains from field 1, S2 and G2 seeds and grains from field 2, respectively.

In the meantime Fessel et al. (2006) noticed that the decrease in the corn seeds vigor was directly proportional to the loss of potassium through the damaged membrane, corroborating Vanzolini & Nakagawa (2003) for peanut seeds, Costa et al. (2008) for pea seeds, Fessel et al. (2010) for soy seeds and Silva et al. (2012) for bean seeds.

By relating the present results with the germination tests performed by Cassol et al. (2016), we can notice that beans from field 1 demonstrated, after 360 days of storage, a lower germination potential. At the same time, they showed greater quantities of potassium release in the electrical conductivity water, even though this relation didn't show the same tendencies during tests. So it isn't possible to associate K^+ amounts with beans vigor loss. Thus we assume that the relationship between the quality of storage seeds and grains and the biochemical behavior is not well established yet, requiring further studies.

Over the course of this research we conclude that it was possible to verify that the variation of the electrical conductivity values didn't show any correspondent relation with the quantification of the investigated ions, indicating that this test wasn't efficient to detect beans vigor decrease, in a way that the amount and behavior of soluble sugars and ions, during storage, didn't allow the correlation between these variables with beans vigor downfall.

CONCLUSIONS

Beans seeds and grains evaluated showed ions modifications, during 360 days of storage, under natural conditions.

The behavior of these parameters, under conditions which the test was performed, was not efficient enough to relate it with the physiological quality and longevity of the BRS Splendor variety.

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RESUMO: O desenvolvimento de pesquisas sobre vigor proporciona maior conhecimento sobre o comportamento fisiológico das sementes ao longo do armazenamento. O objetivo desse estudo foi avaliar as mudanças bioquímicas ocorridas em feijões armazenados por 360 dias. As análises foram realizadas aos 0, 30, 90, 180, 270 e 360 dias, após o recebimento das sementes e grãos da cultivar BRS Esplendor. Foram realizados os testes de teor de água, condutividade elétrica, e a composição química da solução de embebição, onde os teores de açúcares e íons Fe^{2+}, Zn, Ca^{2+}, Mg^{2+} e K^+ foram quantificados. O delineamento foi inteiramente casualizado em esquema de parcelas subdivididas e as médias comparadas pelo teste de Tukey (p>0,05). A condutividade elétrica apresentou maior perda de solutos aos 90 dias. As quantidades de potássio e cálcio diminuíram até os 360 dias, enquanto o magnésio teve aumento após 90 dias de armazenamento. O teste de condutividade elétrica não apresentou relação direta com a quantificação dos íons investigados. A quantidade e o comportamento dos aminoácidos e dos íons estudados não demonstraram relação com a queda do vigor dos feijões.

PALAVRAS-CHAVE: *Phaseolus vulgaris* L. Vigor condutividade elétrica. Lixiviação de eletrólitos.

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