ELECTRICAL CONDUCTIVITY AND MINERAL COMPOSITION OF THE IMBIBITION SOLUTION OF BEAN SEEDS DURING STORAGE

Condutividade elétrica e composição mineral da solução de embebição de sementes de feijão durante o armazenamento

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ABSTRAT

The electrical conductivity test has been used to evaluate seed vigour in many species, especially legumes, such as peas, beans and soybeans. The objective of this research was to evaluate the effect of temperature and storage period on the results of the electrical conductivity test and the mineral composition of the imbibition solution of bean seeds (*Phaseolus vulgaris* L.). Three seed lots of the cultivar IAPAR – 81 and cultivar IPR – TIZIU were stored at 10, 25 and 25-10º C (six months at 10º C and six months at 25º C) The following evaluations were made every three months for a year: seed water content, germination and vigour (accelerated ageing and electrical conductivity) and the potassium, calcium and magnesium contents of the imbibition solution. The cultivars showed similar behavior in storage. The reduction in the vigour of bean seeds stored at 10º C was verified by accelerated ageing and electrical conductivity tests. The bean seed storage at low temperature (10º C) does not influence the results of the evaluation of seed vigour by electrical conductivity test and the quantification of calcium, magnesium and potassium ions. The evaluation of vigour by the electrical conductivity test is not recommended for bean seed stored by long periods (above 9 months).

Index terms: *Phaseolus vulgaris* L, vigour, leaching, low temperature.

RESUMO

O teste de condutividade elétrica tem sido usado para avaliar o vigor de sementes de muitas espécies, especialmente de fabáceas, como ervilha, feijão e soja. Assim, nesta pesquisa, objetivou-se avaliar o efeito da temperatura e do período de armazenamento sobre os resultados do teste de condutividade elétrica e da composição mineral da solução de embebição de sementes de feijão (*Phaseolus vulgaris* L.). Foram utilizados três lotes do cultivar IAPAR – 81 (grupo “Carioca”) e três do cultivar IPR – TIZIU (grupo “Preto”) armazenados a 10, 25 e 25-10º C (seis meses a 10º C e seis meses a 25º C). Ao longo de 12 meses de armazenamento, a cada três meses foram determinados: teor de água, germinação e vigor (envelhecimento acelerado e condutividade elétrica) e conteúdo de potássio, cálcio e magnésio da solução de embebição. As sementes das cultivares apresentaram comportamento semelhante no armazenamento. A redução no vigor das sementes de feijão armazenadas a 10º C foi verificada pelos resultados dos testes de envelhecimento acelerado e de condutividade elétrica. O armazenamento de semente de feijão em baixa temperatura (10º C) não influencia os resultados da avaliação do seu vigor pelo teste condutividade elétrica e a quantificação de cálcio, magnésio e potássio. A avaliação do vigor pelo teste de condutividade elétrica não é recomendada para semente de feijão armazenada por longos períodos (acima de 9 meses).

Termos para indexação: *Phaseolus vulgaris* L, vigor, lixiviação, baixa temperatura.

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INTRODUCTION

An evaluation of seed vigour is important for identifying possible differences in potential performance between similar germination lots (MARCOS-FILHO, 1999). These vigour tests, using direct or indirect methods, estimate the probable behavior of the seed when deterioration occurs, either based on the actual metabolic activity or of constituent parts of the seed (BAALBASKI et al., 2009).

Included among the most well-known vigour tests, electrical conductivity is recommended as a procedure for evaluating pea seed vigour and is suggested for soybean seeds. It also appears promising as a means of standardization (HAMPTON; TEKRONY, 1995; VIEIRA; KRYZANOWSKI, 1999; MARCOS-FILHO; VIEIRA, 2009).

The test measures the quantity of electrolytes liberated during cell membrane restructuring during the

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seed imbibition process. Thus, higher electrolyte levels leached by seeds characterize less vigourous seed lots (VIEIRA, 1994; HAMPTON; TEKRONY, 1995; VIEIRA; KRYZANOWSKI, 1999). Among the solutes leached into the imbibition solution are carbohydrates, fatty acids, amino acids, organic acids, proteins, phenolic compounds and K^+, Ca^{2+}, Na^+ and Mg^{2+} ions (CUSTÓDIO; MARCOS-FILHO, 1997; FESSEL et al., 2006; PANOBIANCO; VIEIRA, 2007; VIEIRA et al., 2008).

The results of the electrical conductivity test can be affected by variables in the methodology, and also other factors, such as genotype (SHORT; LACY, 1976; PANOBIANCO; VIEIRA, 1996; VIEIRA et al., 1996), seed water content (LOEFFLER et al., 1988; VIEIRA et al., 2002) and the stage of seed development (STYER; CANTLIFFE, 1983; POWELL, 1986) among others (MARCOS-FILHO; VIEIRA, 2009). More recent studies showed that the storage conditions, principally low temperatures (10º C), can compromise the reliability of the results, as has been observed in the seeds of soybean (VIEIRA et al., 2001, 2008; PANOBIANCO; VIEIRA, 2007; FESSEL et al., 2010) and peas (PANOBIANCO, et al., 2007). However, this was not seen in corn seeds (FESSEL et al., 2006).

Although research on standardizing the electrical conductivity test for various species has produced important results on procedures, the behavior to different storage conditions for bean seeds, such as low temperatures, have still not been studied. Therefore, the objective of the present research was to evaluate the effect of temperature and storage period on the results of the electrical conductivity test and the mineral composition of the imbibition solution of bean seeds.

MATERIALS AND METHODS

This research was performed in the Seed Analysis Laboratory (Department of Crop Science) and in the Soil Fertility Laboratory of UNESP, Campus of Jaboticabal. Seed lots of two bean cultivars (*Phaseolus vulgaris* L) were used: IAPAR-81 of the "Carioca" group and IPR – TIZIU of the "Preto" group, using three seed lots for each cultivar.

Seeds from each lot were sampled for an initial evaluation and then subdivided into 250 g batches, placed in plastic bags (15 x 30 cm), sealed and stored at different temperatures: 10º C (cold room); 25º C (germination chamber) and 25-10º C, that is, six months in a ermination chamber and six months in a cold room. The physiological potential of the seed lots was evaluated initially and also after three, six, nine and 12 months of storage, using the germination, accelerated ageing and electrical conductivity (EC) tests. The seed water content and mineral composition (Ca^{2+}, Mg^{2+} and K^+) of the soaked seed solution of the EC test were also evaluated. **Seed Water Content (SWC)**: determined by the oven method at 105 ± 3º C for 24 hours, using two replications of approximately 20 g per sample. The data were expressed as a percentage (BRASIL, 2009). **Germination (G):** four replications of 50 seeds were sown in a rolled paper towel, moistened with water equivalent to 2.5 times the weight of the dry substrate and placed in a germination chamber at 25º C. Evaluations were made four and eight days after sowing, according to criteria established by RAS (BRASIL, 2009). **Vigour – accelerated ageing test (AA):** carried out by the plastic germination box method (MCDONALD; PHANEENDRANATH, 1978), using the boxes as individual compartments (mini-chambers), containing 40 mL of deionized water (MARCOS-FILHO, 1999). The seeds were distributed on a steel mesh in a single layer to avoid contact with the water. The closed boxes were kept at 42º C for 72 hours. After ageing, the seeds were submitted to the germination test using four replications of 50 seeds and evaluating on the fifth day after sowing (BRASIL, 2009). The seed water content was also determined at the end of the ageing period. **Vigour – electrical conductivity test (EC):** with four replications of 50 previously weighed seeds, placed for imbibition in 75 mL of deionized water in plastic cups (200 mL) and kept at 25º C for 24 hours. The electrical conductivity of the soaked seed solution was measured after the imbibition period using a conductivity meter (DIGIMED CD-21) with a constant electrode 1. The results were expressed in $\mu S.cm^{-1}.g^{-1}$ of seeds (VIEIRA; KRZYZANOWSKI, 1999, MARCOS-FILHO; VIEIRA, 2009). **Quantification of the Ca^{2+}, Mg^{2+} and K^+ ions:** after reading the electrical conductivity, the ion content of the imbibition solution was determined: the reading of the potassium (K^+) ions was done directly using a flame photometer and the reading of the calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, using an atomic absorption spectrophotometer (BATAGLIA et al., 1983; TOMÉ-JUNIOR, 1997). The final result of the ion content was expressed in mg of ions per kg of seeds. **Statistical analysis:** the experimental units were arranged in a completely randomized design in a 4 x 3 factorial arrangement [four storage periods (three, six, nine and 12 months) x three temperatures (10, 25 and 25-10º C] with an additional treatment (initial evaluation) and four repetitions. Data were submitted to ANOVA and polynomial regression equations were adjusted to describe the behavior of the seeds along the storage periods, for each temperature.

RESULTS AND DISCUSSION

Differences in physiological potential were observed for germination and vigour, from accelerated ageing in the initial evaluation for both cultivars. The electrical conductivity test and the quantification of leached ions detected lot differences only for the IPR-TIZIU cultivar, confirming the results obtained from the other tests, with lot 2 showing the lowest physiological potential. Lot 2 for the IAPAR-81 cultivar also showed a lower physiological quality for the germination and vigour-accelerated ageing tests, but this difference was unclear from the electrical conductivity test and the quantification of the imbibition solute ions. The seed water contents of the lots were similar, with a maximum variation of 0.5 percentage point among lots of the IAPAR-81cultivar and 0.4 percentage point for the IPR-TIZIU cultivar (Table 1).

Considering the amount of information collected, only the storage results for lots 1 and 2 for both cultivars have been shown, due to the difference in physiological potential between the lots, with lot 2 being higher than lot 1, and also to the similarity between the results obtained for lots 3 and 1.

The germination test did not detect any variation in the physiological performance of the seeds of the two cultivars which had higher physiological quality (lot 1), independently of the storage conditions. However, the percentage germination fell significantly during the storage for most of the seeds from lower physiological quality (lot 2). Only seeds from the IPR-TIZIU cultivar stored at 10º C maintained the same germination level (Figures 1 and 2).

The results of the accelerated ageing test showed that seeds stored at 10º C demonstrated small or no changes in vigour during storage (Figures 1 and 2), except for the seed lot with a lower physiological quality of the IPR-TIZIU cultivar, which showed a significant reduction in seed vigour, even when stored at this temperature. When stored at 25 and 25-10º C, an increase in the seed deterioration process was observed during storage, based on the tendency of reduced germination after the accelerated ageing test for the two lots of both cultivars (Figures 1and 2).

The electrical conductivity test showed small reduction in the seed vigour of two lots of the IAPAR- 81 cultivar stored at 10º C and the lot 1 stored at 25/10º C. For the IPR-TIZIU cultivar, the electrical conductivity test detected little variation in the seed vigour of the higher quality lot during storage and did not detect any differences between storage temperatures. However, for the seed lot with lower physiological quality, the electrical conductivity results show that storage at 10º C allowed better conservation of seed vigour after six months (Figure 2). A small reduction in the electrical conductivity values was observed for the last evaluation.

The ion most leached for the two cultivars was potassium, followed by magnesium and, in smaller quantities, by calcium. The values for potassium corresponded most closely to the results of the electrical conductivity test for the lots of cultivar IAPAR – 81, except for the last evaluation (Figure 3). The quantity of these ions in the seeds stored at 25 °C increased with the storage time (Figure 3).

The content of Ca^{++}, Mg^{++} and K^{+} ions leached into the seed imbibition solution of the seed lots of cultivar IPR-TIZIU, provide similar results to those of the electrical conductivity test. Small variations in ion leaching during storage were observed for seeds with a higher physiological quality (lot 1) for all temperatures. However, for seeds with a lower physiological quality (lot 2), mainly the potassium ion showed tendency to increase during storage (Figure 4).

Table 1 – Seed water content (SWC), standard germination (SG), accelerated ageing (AA), electrical conductivity (EC) and contents of potassium, calcium and magnesium in the soaked water of bean seeds, IAPAR-81 and IPR-TIZIU cultivars, at initial evaluation.

Cultivar	Lot	SWC	SG	AA	EC	Ca^{++}	Mg^{++}	K^{+}
		-------------%---------			$\mu S\ cm^{-1}\ g^{-1}$	---------------mg kg^{-1}--------------		
IAPAR-81	1	12.5	98	93	105	36.7	130.9	2287.9
	2	13.0	90	69	99	34.0	127.6	2220.3
	3	12.7	98	97	103	31.0	130.3	2263.1
IPR-TIZIU	1	12.4	98	93	67	30.5	115.5	1453.0
	2	12.7	81	42	117	43.0	215.4	3142.2
	3	12.8	92	58	93	36.7	171.7	2330.6

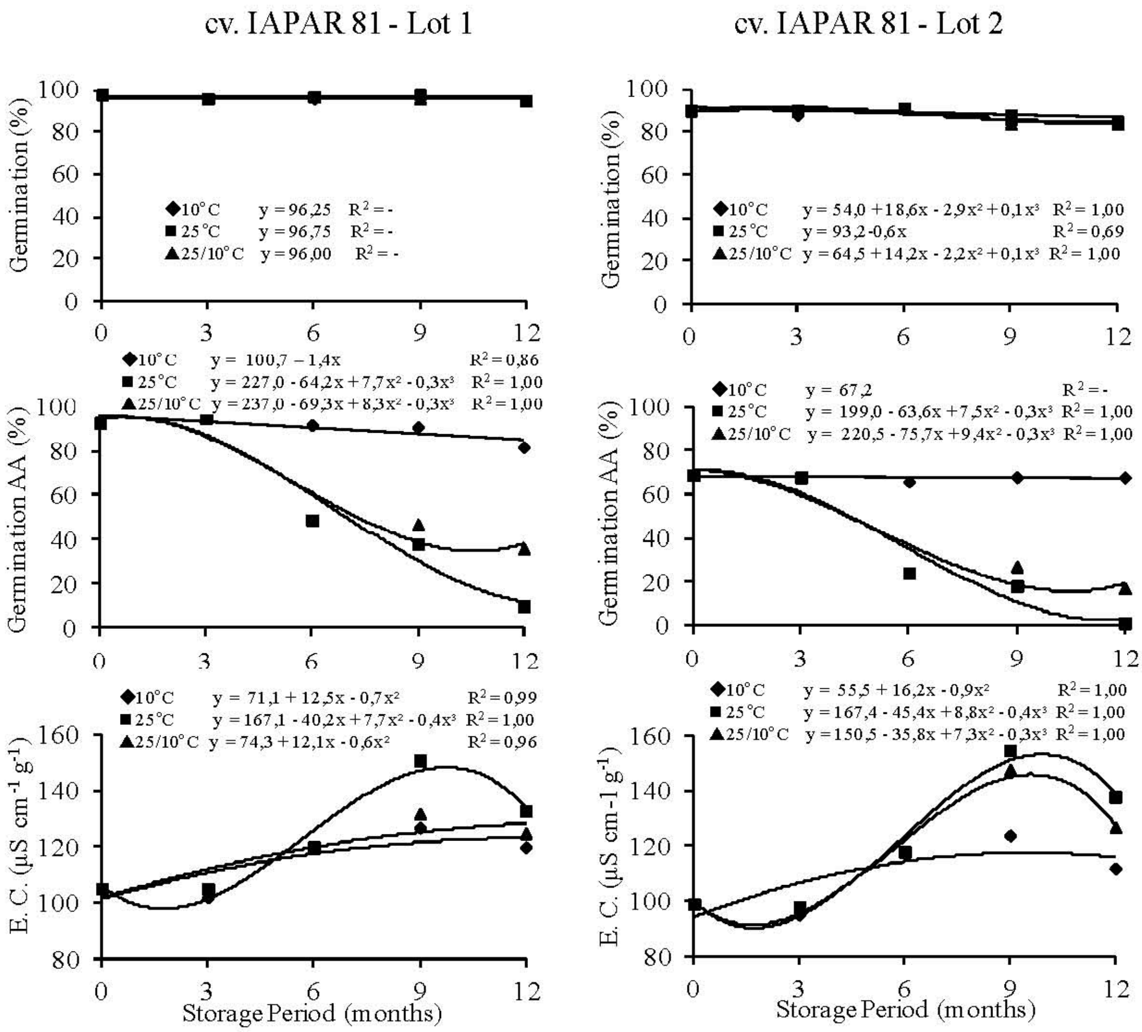

Figure 1 – Germination and vigour evaluated by accelerated ageing (AA) and electrical conductivity (E C) of bean seeds, cultivar IAPAR-81, lots 1 (high vigour) and 2 (low vigour), during storage at three temperatures.

A general analysis of the data showed that deterioration occurred for all storage conditions since this process is inevitable and continuous (MARCOS-FILHO, 2005), but the physiological quality of bean seeds stayed at higher levels when stored at the lowest temperature (10º C), independently of the cultivar. A low storage temperature reduces the speed of deterioration once seed metabolism is lowered (MARCOS-FILHO, 2005).

The fall in the vigour of bean seeds stored at low temperatures, 10º C and initially at 25º C and transferred to 10º C after six months, could be seen from the results of the accelerated ageing test and also from the electrical conductivity and ion leaching, principally potassium. This indicates that the initial stages of bean seed deterioration at 10º C storage may be related to the continuous destructuring and inefficiency of the restructuring mechanisms of the cellular membrane systems storage at and that her vigour can be evaluated by the electrical conductivity test, because this test supplies the same results that the accelerated ageing test. Similar results were observed in corn seeds (FESSEL et al., 2006), where low storage temperatures did not affect the electrical conductivity results.

In the case of beans, a reduction tendency in the electrical conductivity values starting from nine months of storage was probably due to a "*hardshell*" of the beans during storage, a common phenomenon widely studied in food technology. This phenomenon is characterized by coating impermeable to water. An increase in tegument impermeability during storage can result in lower electrical conductivity values for the imbibition solution, upsetting the expected results of the vigour test (ARAGÃO et al., 2000).

Thus, a reduction in the electrical conductivity values may be explained by an increase in tegument impermeability, although the leaching of calcium, magnesium and potassium ions mostly did not show the same reduction tendency, which may indicate that other cellular solutes (carbohydrates, fatty acids, amino acids, organic acids, proteins, phenolic compounds) can also affect the electrical conductivity results for the bean seed imbibition solution (CUSTÓDIO; MARCOS-FILHO, 1997; VIEIRA; KRZYZANOWSKI, 1999; FESSEL et al., 2006; PANOBIANCO; VIEIRA, 2007, VIEIRA et al., 2008)

The protein and amino acid contents in the bean seed may influence the results of the electrical conductivity test, masking the results of the vigour analysis test of stored seeds, since the reduction of the contents, or their solubility due to the deterioration process during the storage, can reduce the electrical conductivity values of the imbibition solution of the stored bean seeds.

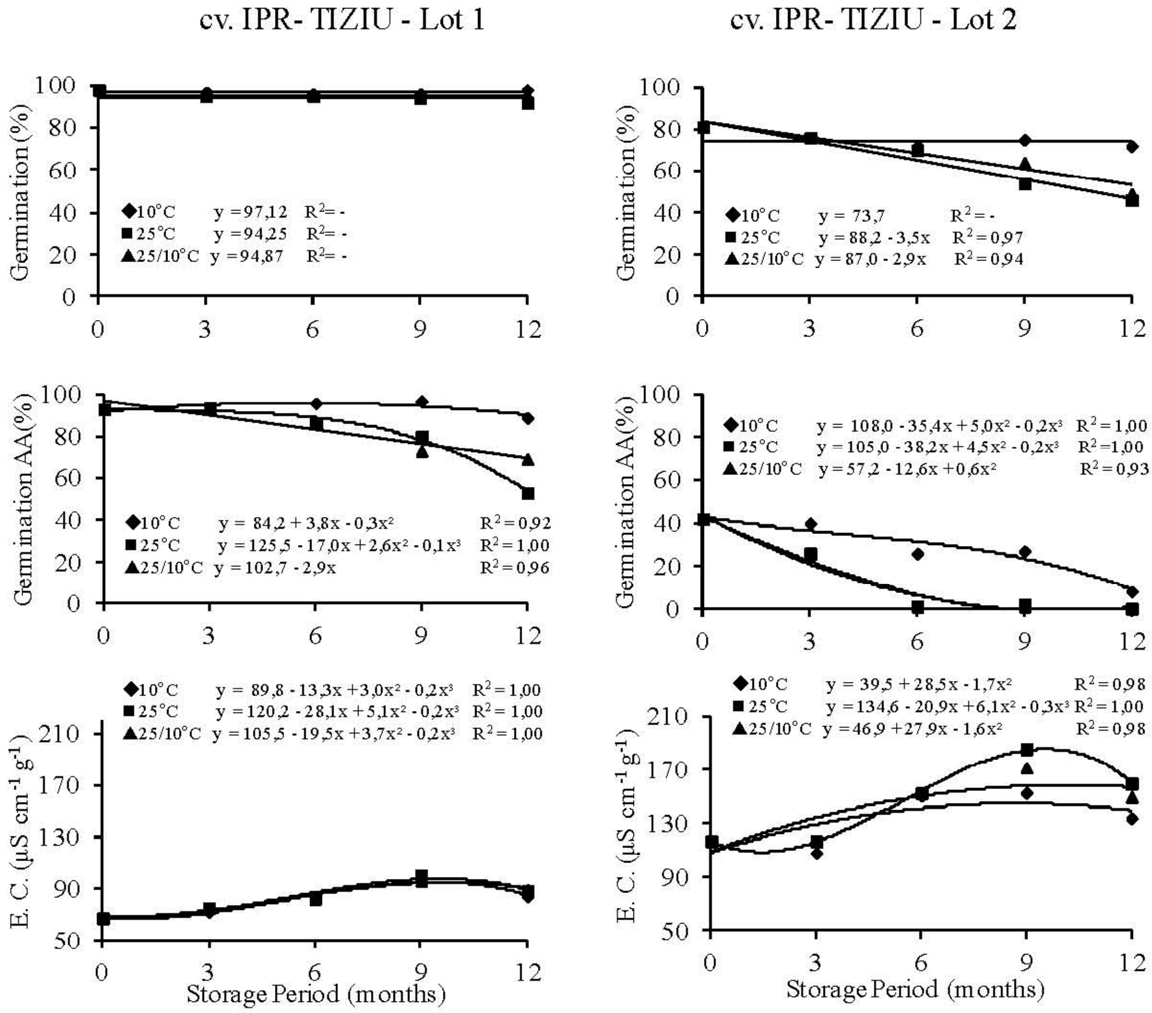

Figure 2 – Germination and vigour evaluated by accelerated ageing (AA) and electrical conductivity (E.C) of bean seeds, cultivar IPR-TIZIU, lots 1 (high vigour) and 2 (low vigour), during storage at three temperatures.

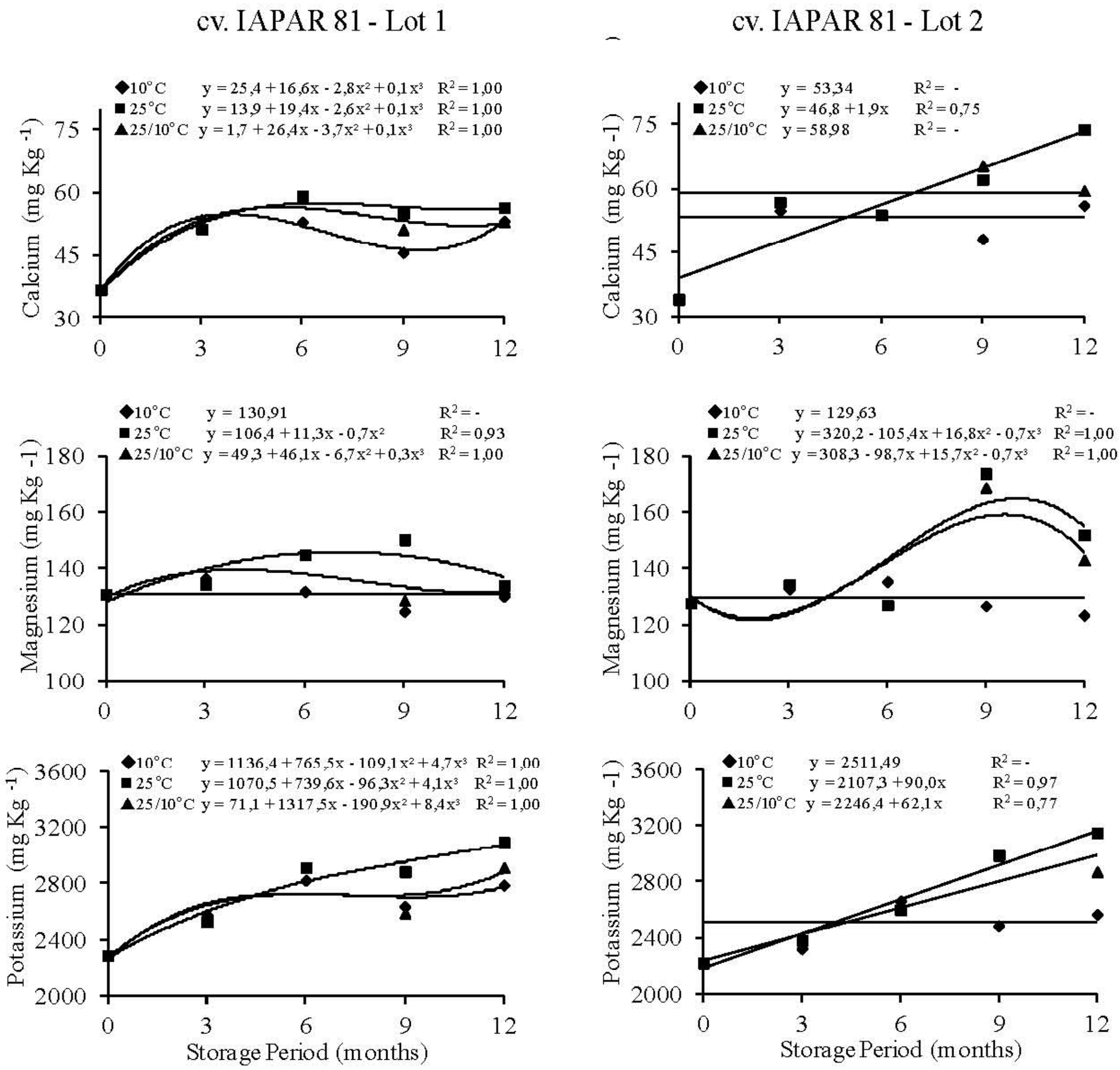

Figure 3 – Contents of calcium, magnesium and potassium in the soaked water of bean seeds, cultivar IAPAR-81, lots 1 (high vigour) and 2 (low vigour), during storage at three temperatures.

Most of the leachates in the imbibition solution from the conductivity test in bean seeds consist of amino acids, proteins and sugars, perhaps because there are greater amounts of them in the seeds, since proteins and amides are the main reserves (BINOTTI et al., 2008). Also, during seed ageing there are changes in the quantities of reserve compounds. After ageing, a reduction in the protein content (BEGNAMI; CORTELAZZO, 1996) and also its solubility (RIBEIRO et al., 2005), may occur.

With the obtained results it is possible to observe that the storage period above nine months can have larger influence on the results of the evaluation of seed vigour for electric conductivity test than the temperature during the storage owed the physical and chemical alterations that happen in bean seeds during the storage, independent of the storage temperature.

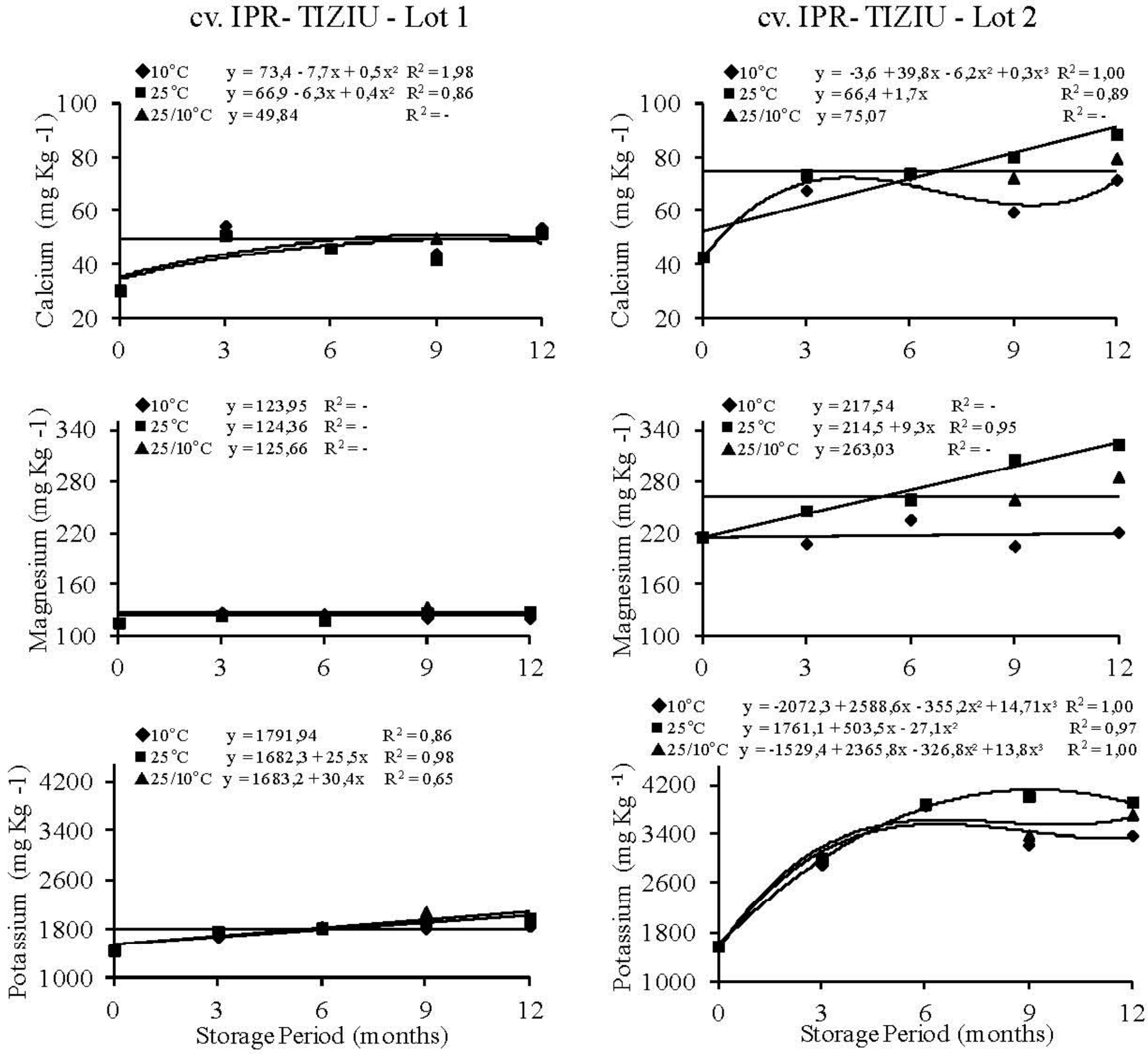

Figure 4 – Contents of calcium, magnesium and potassium in the soaked water of bean seeds, cultivar IPR-TIZIU, lots 1 (high vigour) and 2 (low vigour), during storage at three temperatures.

CONCLUSIONS

Bean seed storage at low temperature (10°C) does not influence the results of the evaluation of seed vigour by electrical conductivity test and the quantification of calcium, magnesium and potassium ions.

The evaluation of vigour is not recommended, for the electrical conductivity, for bean seed stored by long periods (above 9 months).

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