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## Image analysis and peanut seeds performance during the production process

### Análise de imagens e desempenho de sementes de amendoim durante o processo de produção

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#### Abstract

During the production process, obtaining high quality seeds is subject to careful management and quality assessment procedure for the stages process. This work aimed to verify the automated system of image analysis (SVIS<sup>®</sup>) efficiency to evaluate the peanut seeds physiological potential obtained during the production process. Treatments consisted of plant digging, combine, transportation, drying, storage (two, four, and six months), and the following processing steps: mechanical threshing, classification by size, separation by density and color, and chemical treatment. The seeds were evaluated by moisture content, germination, vigor and SVIS<sup>®</sup>. Data were analyzed by uni and multivariate statistical procedures. During harvest until storage stages, the seeds had high physiological potential. This good quality was not sustained with the following stages. Results of SVIS<sup>®</sup> analyses, along with other tests, were highly correlated with the new latent variable obtained by principal component analysis. Results provided by SVIS<sup>®</sup> has strong relationship with the other procedures to evaluate the physiological potential of peanut seeds from different stages of the production process.

**Additional keywords:** *Arachis hypogaea* L.; principal component; processing; storage; vigor.

#### Resumo

Durante o processo de produção, a obtenção de sementes de elevada qualidade está condicionada ao manejo cuidadoso e ao procedimento de avaliação da qualidade durante as diferentes etapas. Dessa forma, este trabalho teve por objetivo verificar a eficiência do sistema automatizado de análise de imagens (SVIS<sup>®</sup>) para a avaliação do potencial fisiológico de sementes de amendoim obtidas durante o processo de produção. Os tratamentos consistiram em sementes obtidas após as etapas de arranquio das plantas, recolhimento, transporte, secagem, armazenamento após dois, quatro e seis meses, beneficiamento, após a trilha mecânica, classificação por tamanho, separação por densidade, coloração e após o tratamento químico, as quais foram avaliadas quanto ao teor de água, germinação, vigor e pelo SVIS<sup>®</sup>. Os dados foram analisados por procedimentos estatísticos uni e multivariados. Durante as etapas da colheita ao armazenamento, as sementes apresentaram elevado potencial fisiológico, que não se manteve com o acúmulo das etapas seguintes. Os índices de SVIS<sup>®</sup>, juntamente com os demais testes, apresentaram elevada correlação com a nova variável latente obtida pela análise de componentes principais. Os índices fornecidos pelo sistema automatizado de análise de imagens (SVIS<sup>®</sup>) têm forte relação com os demais procedimentos para a avaliação do potencial fisiológico de sementes de amendoim provenientes de diferentes etapas do processo de produção.

**Palavras-chave adicionais:** *Arachis hypogaea* L.; armazenamento; componentes principais; processamento; vigor

#### Introduction

The success of any producing crop depends, among other factors, the use of seeds of high and proven physical, physiological and sanitary quality. In the case of peanut seeds, it is common to obtain seeds with low physiological quality regardless the technology level used for production.

Some peanut cropping characteristics support the production of seeds with low physiological quality: later maturation of seeds (Balota & Phipps, 2013), which leads harvest during the rainy season, and injures mechanized operations and natural drying or "cure" (Haro et al., 2008), indeterminate plant development (Branch et al., 2010), high contamination by pathogens (Barbosa et

al., 2013a), besides problems during processing (Barbosa et al., 2014).

The identification and correction of the points that reduce the physiological quality of seeds, during the production process of peanut seeds, is crucial for obtaining lots which have the minimum standards for commercialization. Thus, appropriate procedures for the physiological potential analysis are essential for the correct identification of problems during the production process of seeds. Therefore, only the germination test provides limited information about the quality of seeds as it is conducted in great conditions of temperature, water availability and oxygen (Barbosa et al., 2012). It should be considered that vigor tests provide more sensitive indexes of the physiological potential in order to complement information.

It is understood by vigor of seeds as the set of properties that determine the potential of seed lots, with acceptable germination, for rapid and uniform seedling emergence, under wide range of environmental conditions (Tekrony, 2003).

With the advent of new technologies, the evaluation of seed physiological potential, computerized methods have been developed and are available. Thus, to evaluate the vigor of lettuce seeds an automated system was developed named Seed Vigor Imaging System (SVIS®), which measures vigor, uniformity of development and seedling growth indexes (Sako et al., 2001). This procedure has been used to evaluate the physiological potential of several species seeds, such as soybean (Marcos-Filho et al., 2009), crotalaria (Silva et al., 2012) and bean (Gomes-Junior et al., 2014). The method has been successfully tested for evaluate the physiological

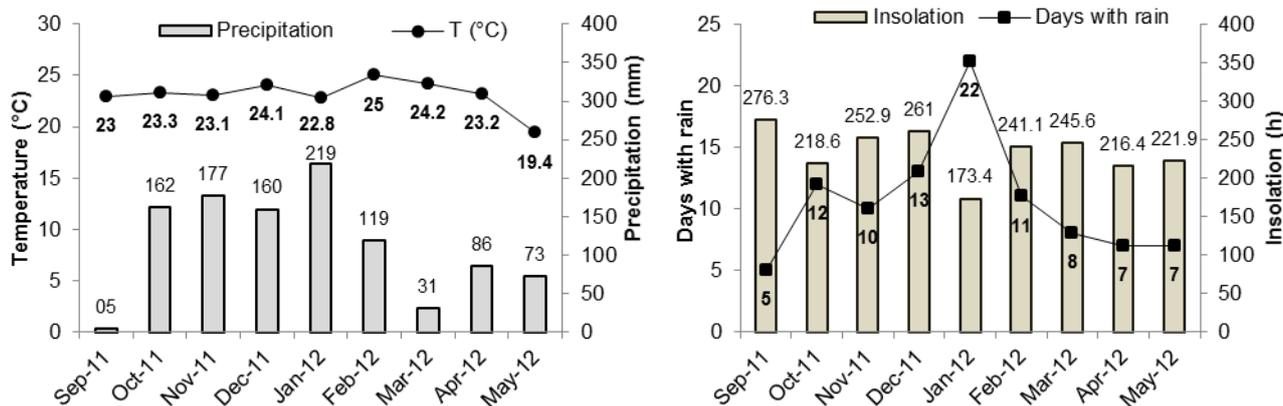
potential of peanut seeds treated with fungicide and insecticide (Marchi et al., 2011). The SVIS® was used in these studies to evaluate the physiological potential of seeds compared with the results of traditional tests. However, considering that each vigor test evaluates seeds of a different way, either by tests under stress, seedling performance or even by biochemical tests, it is necessary that tests of germination and vigor are together analyzed for the characterization of physiological potential of seed lots (Barbosa et al., 2013b).

Thus, the aim of this study was to verify the automated system efficiency of image analysis (SVIS®) compared to others tests for the physiological potential evaluation of peanut seeds obtained during the production process.

### Material and methods

Seeds were obtained from the cv. Runner IAC 886 peanut production field (Virginia group), under the responsibility of the Cooperativa dos Plantadores de Cana da Zona de Guariba (COPLANA). In the crop year 2011/2012, the field was under the coordinates 21°14'15"S and 48°21'09"W, at 660 m of altitude, located within the boundaries of the municipality of Jaboticabal, State of São Paulo.

During the conducted period of crop, climatological data, average air temperature (°C), rainfall (mm) insolation (hours) and number days with rain, were recorded by the Agrometeorological Station of the Faculdade de Ciências Agrárias e Veterinárias, UNESP, Campus of Jaboticabal (Figure 1).



**Figure 1** - Ambient conditions for the period of field implementation and conduct peanut 'Runner IAC 886' seed production: (a) rainfall (mm) and average air temperature (°C); (b) insolation (hours) and days with rain, for the crop year 2011/2012. Source: Agrometeorological station, FCAV, Unesp, Jaboticabal.

Peanut seeds were sampled in the field and at the Seed Processing Unit (SPU). For each step of the production process, four simple samples were obtained that after homogenization, formed the composite sample, which later was referred to the Seed Analysis Laboratory of the Department of Plant Production, UNESP. Seeds from the harvest stages were

manually threshed until the end of storage and after each processing stage carried out at SPU, seeds already peeled were obtained.

In Table 1, the stages, also named treatments, whose seeds were subjected during the production process, have been described.

**Table 1** - Treatments from different stages of peanut seed production process 'Runner IAC 886' in 2011/2012.

Stage	Description
T1	After mechanical digging and natural cure.
T2	After mechanized gathering, with sampling being performed with the grain tank of the harvester with the pods already disconnected from plants in the field.
T3	After field transportation to SPU, seeds are still pods, newcomers to SPU, before drying process.
T4	Pods after drying process conclusion, in which the pods were pre-cleaning, drying, and cleaning; before bagging.
T5	Sampling after two months of storage: pods stored in 200 kg polyethylene bags at warehouse environment condition (25 °C air temperature and 60% of relative air humidity).
T6	Sampling after four months of storage.
T7	Sampling after six months of storage.
T8	After mechanical threshing, in processing.
T9	After size classification, in which seeds of the sieve 23 were collected.
T10	After separation by density of the seeds by gravity table.
T11	After coloration selection by electronic selector.
T12	After the commercial chemical treatment of seeds.

Samples of each treatment were properly identified and kept in dry chamber (25 °C and 40% of relative air humidity), without chemical treatment with fungicides or insecticides, until assessments, except T12.

The commercial chemical treatment (T12) consisted of 200 mL of p.c. fludioxonil + metalaxyl fungicide application, commercial product in 100 kg of seeds; thiamethoxam insecticide, 150 mL of p.c. in 100 kg of seeds. For the micronutrients supply, product with concentration of 1.5% cobalt and 14% of molybdenum, at 200 mL of p.c. dose, in 100 kg of seed and commercial polymer for the treatment and seed coating in 200 mL dose at 100 kg of seeds has been used.

After manual processing, seeds from the treatments T1 to T7, were classified by size and those retained were used in the round sieve of 23 mm. After obtaining seeds of the other treatments, T8 to T12, these were subjected to water content determination and germination evaluations, first count, emergence speed index, accelerated aging, electrical conductivity, tetrazolium, seedling emergence in the field, besides the automated system for seed vigor evaluation (SVIS®).

The water content was carried out with two replications of 25 seeds in greenhouse at  $105 \pm 3$  °C, for 24 h. (Brazil, 2009) and results expressed in percentage (wet basis).

The germination test was conducted with eight replicates of 25 seeds of each stage, previously chemically treated with fungicide thiram (500 dF<sup>-1</sup> of active ingredient) in a 300 mL dose of commercial product per each 100 kg of seeds (Brazil, 2009; Barbosa et al., 2013a) and distributed on wet germination paper rolls with amount of deionized water equivalent to three times of dry substrate mass and placed in a growth chamber at 25 °C with 12 h photoperiod. Evaluations were performed in the fifth day, corresponding to the first

count of germination, and at the tenth day after sowing, adding to the percentage of normal seedlings (Brazil, 2009).

Emergency speed index (ESI) was obtained from another germination test, on sand. In this case, as described, chemically treated seeds were sown in plastic boxes containing sand as substrate with water equivalent to 60% of its holding capacity. Evaluations were performed through daily counts of emerged seedlings, considering those whose cotyledons reached the substrate level. Later the seedling emergence speed index was calculated, considering the sum of the ratio between the numbers of emerged seedlings on the number of days that it took to emerge (Maguire, 1962).

For accelerated aging, samples with approximately 250 seeds of each treatment, chemically treated for germination as described, were distributed into single uniform layer over stainless steel screen and placed in plastic boxes (11x11x3.5 cm), with 40 mL of deionized water at the bottom. Boxes were maintained in germination chamber at 42 °C for 72 h (Marcos-Filho, 1999). Subsequently, eight replicates of 25 seeds were submitted to the germination test (Brazil, 2009), and at the fifth day after sowing, the assessment was performed.

For electrical conductivity, four replicates of 50 seeds from each treatment were weighed and placed to soak in plastic cups (200 mL capacity) containing 75 mL of deionized water at 25 °C for 24 h. After this time, solution electrical conductivity was measured and the results expressed as  $\mu\text{S cm}^{-1} \text{ g}^{-1}$  (Barbosa et al., 2012).

The tetrazolium test was performed with four replicates of 25 seeds of each treatment, preconditioned for 16 h at 25 °C by immersion in water. Afterwards, cotyledons were separated with the aid of blade and immersed in a solution of 2,3,5-triphenyl tetrazolium chloride at 0.075% for 2 hours at 40 °C.

Results were expressed as percentage of viable seeds (Santos et al., 2012).

For seedling emergence in the field, four replications of 50 seeds were sown chemically treated for germination as described, in furrows of 2 m length and 3-4 cm depth. Furrows were 0.4 m spaced and evaluated after 15 days, counting the seedlings whose primary leaves were completely developed.

Computerized analysis of seedlings (SVIS®) was performed with four replications of 50 seeds for each treatment. Seeds were divided into 25 groups (five rows of five seeds) in paper sheets for germination, moistened with water equivalent to three times their dry mass and placed in a germinator at 25 °C with 12 h photoperiod. Acquisition of images was carried out on the fifth day after test installation. Images of normal and abnormal seedlings or dead seeds were obtained by scanner HP Scanjet 2,400 with 30x21 cm scanning area, installed in an inverted position, inside the wood box (60x50x12 cm), adjusted in resolution of 100 dpi and coupled to a Core i5 computer (6 GB of RAM and 500GB HD). After image processing which were performed at the Laboratory of Image Analysis of the Department of Plant Production, Universidade de São Paulo, the vigor (VI), growth (GI) and growth uniformity (GU) indexes were generated. The vigor index is the result from the combination between growth and uniformity indexes, which can vary values from minimum zero to maximum 1,000. Data were obtained setting as 4.0 cm the maximum size of seedling. In order to obtain the vigor index, the following composition was used:  $IV70/30 = \{(0.7 \times IC) + (0.3 \times IU)\}$ . When necessary, small adjustments were carried out, properly to recognize and to determine the connection point between the primary root and the shoot of normal seedlings (Marchi et al., 2011).

Data were tested concerning normality (Anderson-Darling test) and homogeneity of variances, and by attending the relevant requirements of variance analysis, were not processed. Later, they were subjected to variance analysis in randomized experimental design, with 12 treatments and four replications. Means were compared by the Scott-Knott test at 5% probability (Pimentel-Gomes, 2009).

In order to verify the relationship between the assessments of the physiological potential of peanut seeds, the multivariate principal component analysis was performed after the variables standardization, in which each variable had mean 0 and variance 1. The principal component analysis allows condense the most amount of original information contained in  $p$  variables ( $p = 10$ , this study) in two orthogonal latent variables named principal components, which are linear combinations of the original variables created with the two highest eigenvalues of data covariance matrix (Hair et al., 2005). The appropriateness of this analysis is verified by the amount of total information of original

variables retained by principal components that show higher eigenvalues than the unity (Kaiser, 1958).

All statistical analyzes were performed using the STATISTICA software version 7.0 (Statsoft, 2004).

## Results and discussions

The peanut crop showed 130 days cycle and the digging plants occurred on March 07, 2012. Also in the field, after three days of the digging corresponding to the natural drying or "cure" period, peanut gathering was performed, which is the separation of the pods from plant and their storage in the grain tank of the machine.

In March 2012, environmental conditions were conducive to the peanut "cure" process aimed at producing seeds with high physiological quality. There were only eight days with rain, low rainfall, high temperatures and 245.6 hours of insolation (Figure 1), which together accelerated plants and pods natural dry.

Water content of the seeds from the different stages of the production process, at the time of the evaluation of physiological potential, ranged from 4.2 to 6.2% (data not shown). Water content uniformity among different treatments provides safety in the evaluation of seed quality and it is essential for obtaining consistent results (Vieira et al., 2002).

Seeds from digging stages until the four month of storage (T1 to T6) showed high physiological potential (Figure 2). These seeds had germination with values ranging from 84% to 92%, all above the standard established for commercialization, in the State of São Paulo which is 70%. These same stages still presented high vigor index evaluated by the first count tests of germination (from 75% to 90%), seedling emergence speed index (from 7.9 to 9.1), accelerated aging (from 82% to 90%) and electrical conductivity (from 5.6 to 18.9  $\mu\text{S cm}^{-1} \text{g}^{-1}$ ) (Figure 2a-e). Owing to these stages are close to the physiological maturity point, it is natural that seeds have higher physiological quality (Carvalho & Nakagawa, 2012).

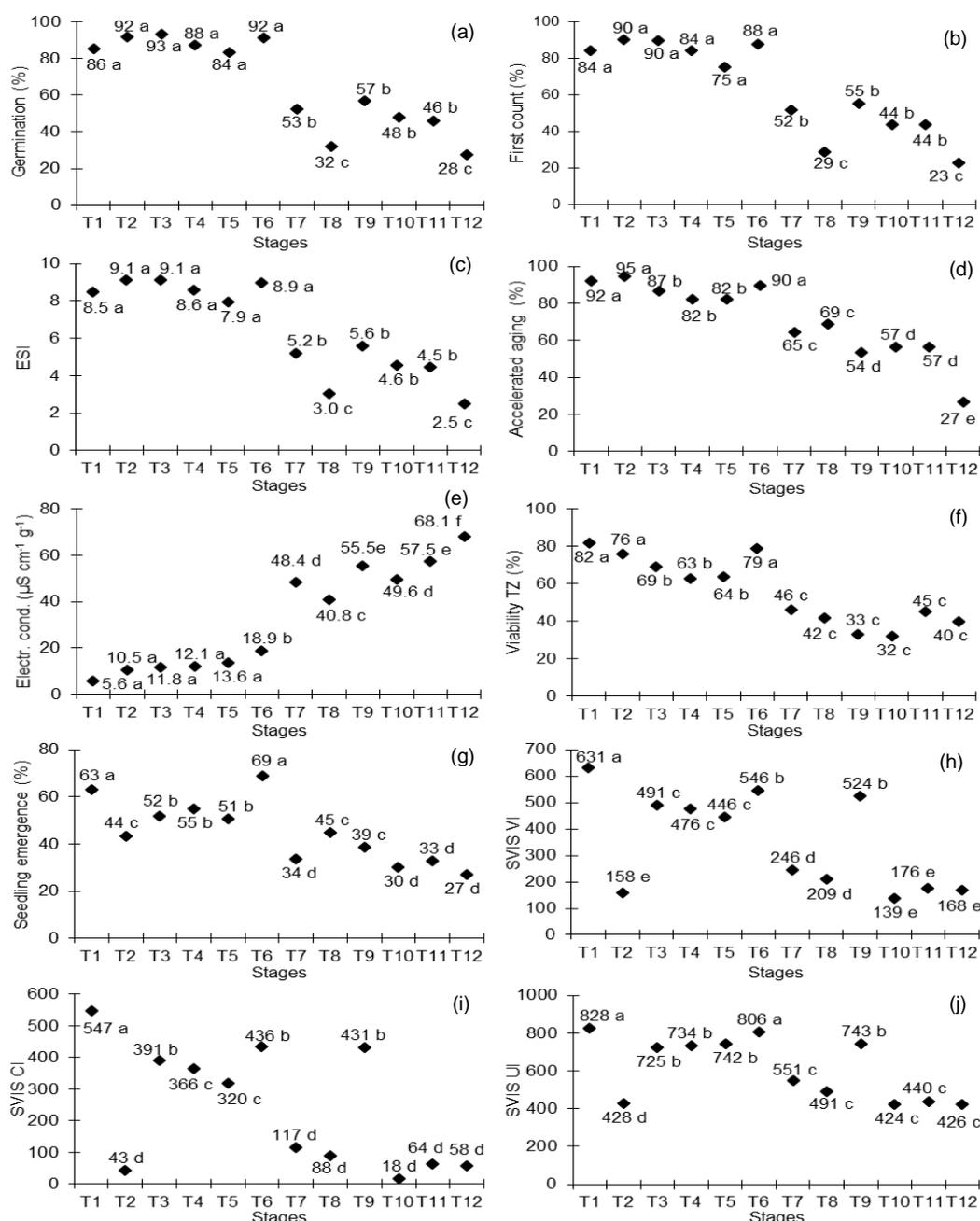
With the end of the storage and the beginning of processing (T7 towards), the physiological potential of seeds decreased during the subsequent stages. Seeds had low germination and vigor, verified by all assessment procedures (Figure 2a-j).

Processing damaged the physiological quality, especially after the operation of mechanical threshing (T8). The friction of the threshing cylinder, used for the extraction of seeds from the pods, can lead to problems for obtaining seeds with high quality.

Thus, processing cannot improve the physiological quality of peanut seeds, and at the end of process seeds without standard for commercialization are obtained. This finding was also observed for 'Tatu Vermelho' (Fessel & Barreto, 2000), 'Runner

IAC 503' and 'Runner IAC 886' peanut seeds (Santos et al., 2013; Barbosa et al., 2014). However, the processing operations are indispensable for obtaining high quality seeds (Barbosa et al., 2014). In this case,

in order to have a product with good quality at the end of the process, changes and management care should be made.



**Figure 2** - Germination, first count of germination, emergence speed index, accelerated aging, electrical conductivity, tetrazolium viability test, seedling emergence in the field and SVIS® (vigor index, growth and uniformity) of peanut seeds 'Runner IAC 886' from different stages of the production process in the agricultural year 2011/2012. Means followed by the same letter do not differ by Scott-Knott test at 5% probability. T1, digging; T2, combine; T3, transport; T4, drying; T5, storage for two months; T6, storage for four months; T7, storage for six months; T8, mechanical threshing; T9, classification by size; T10, separation by gravity table; T11, staining selection; T12 commercial chemical treatment.

Seeds of mechanical threshing stages (T8 to T12) showed low germination values and seedlings emergence in the field for chemical treatment (Figure 2g). In these evaluations, the seed dormancy

hypothesis was discarded, because in the tetrazolium test, has been verified serious injury to the embryonic axis tissue, and also dead seeds (Figure 3). The results of electrical conductivity of the soaking

solution also corroborate this finding. The high values indicate the occurrence of increased release of leached into the solution, due to health problems of cell membranes seeds with low potential of

performance (Barbosa et al., 2012). The membrane degeneration and reduced seedling development are closely associated during the process of seed deterioration (Delouche & Baskin, 1973).



**Figure 3.** Peanuts seeds, cv. Runner IAC 886, from the stage after chemical seed treatment (T12) considered unfeasible by the tetrazolium test.

Regarding the computerized analysis of seedlings, the highest values of vigor, growth and uniformity indexes were obtained for seeds from the treatments obtained after plant digging (T1) (Figure 2 h, i and j). From this moment, results showed decreased quality with the accumulation of stages during the production process.

Information was corroborated by computerized analysis about physiological quality verified by others evaluations, routinely used in the Seed Laboratory. In addition, it also showed a reduction in physiological quality during the different stages of the production process.

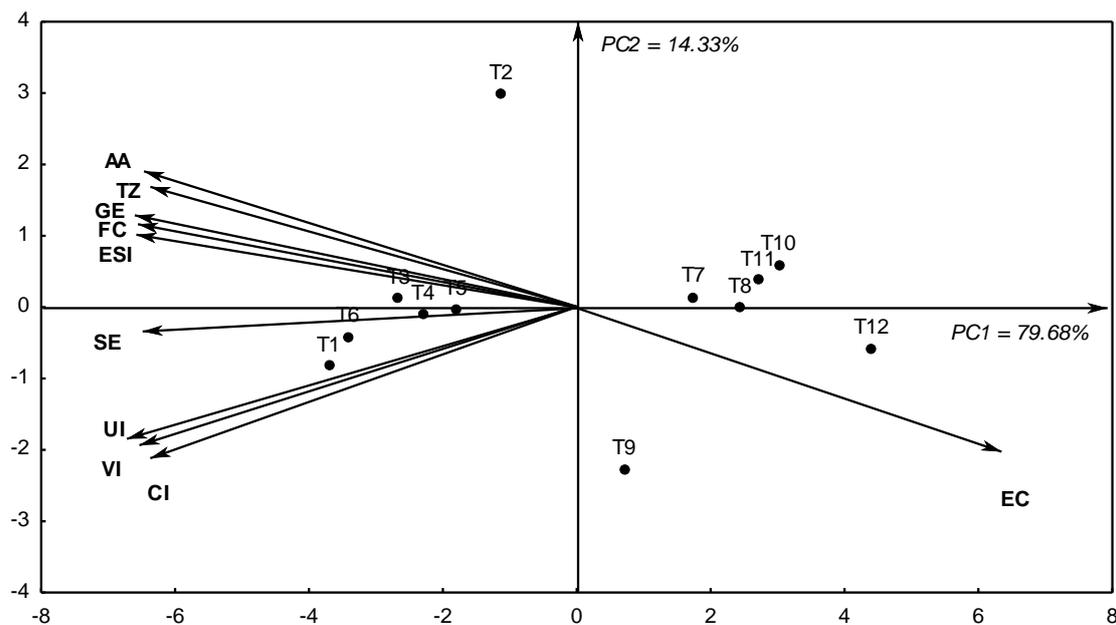
For soybeans, the vigor measured by SVIS® showed comparable results from those obtained in the first count of germination assessments, germination speed index, accelerated aging and seedling emergence in the field (Marcos-Filho et al., 2009). On the other hand, this comparison may provide misinterpretations since univariate analysis may be incomplete, as a set of variables, only multivariate procedures simultaneously evaluate a set of characteristics, considering existent correlations (Santos et al., 2010).

Due to the number of variables and the need to improve the understanding of the relationship between the assessment procedures of the physio-

logical quality of peanut seeds during the production process, the multivariate statistical exploratory technique applied by principal component analysis allowed to discriminate the production process stages more associated with each assessment test of the physiological potential, as well to characterize and to relate the variables responsible for discrimination (Figure 4).

The two principal components (PC1 and PC2) together retained 94% (79.7% in PC1 and 14.3% in PC2) of the original variability (Figure 4). The resulting graphical representation of the principal component analysis revealed the relationship between T1 to T6 treatments and quality assessments related to germination and vigor regarding the performance of seedlings, and the indexes obtained from the computer program SVIS® as well.

The discriminating power of the variables in each principal component is measured by the correlation between each variable and a principal component (Table 2). The high correlation values of all variables show strong relationship with PC1. Negative correlations were responsible for the discrimination of the treatments derived from the cropping seeds until the storage for four months (T1 to T6), located to the left in PC1 (Figure 4).



**Figure 4.** Dispersion of treatments obtained during the process of production of peanut seeds 'IAC886 Runner' and its relations with the evaluation of physiological seed quality. GE, germination; FC, first count of germination; ESI, emergency speed index; AE, accelerated aging; EC, electrical conductivity; TZ, tetrazolium viability test; EM, seedling emergence in the field; and SVIS® (VI, vigor index, GI, growth, UI, uniformity); T1, digging; T2, combine; T3, transport; T4, drying; T5, storage for two months; T6, storage for four months; T7, storage for six months; T8, mechanical threshing; T9, classification by size; T10, separation by gravity table; T11, staining selection; T12 commercial chemical treatment; CP1, principal component 1; CP2, principal component 2.

The high correlation of CP1 with the indexes provided by SVIS® as well the others assessments of seed physiological quality (germination, first count, speed index of accelerated aging and seedling emergence in the field) indicate that these variables retain the maximum possible of information that explain the majority of the total variability, revealing that there is a strong relationship between the variables (Santos et al., 2010).

Thus, seeds derived from T1 to T6 treatments have greater potential to form seedlings under a wide range of environmental conditions, as germination, as well as vigor tests show high results. In addition, the strong relationship between the indexes obtained from the SVIS®, with the others tests for quality assessment revealed that the system shows results comparable to others procedures. Therefore, the research for principal components allowed a better data understanding, and becomes very significant tool to help the assessment of the physiological quality of peanut seeds.

In the second principal component, variables had low discriminatory power of treatments. Thus, by CP2 retaining only 14.33% of the total variability of the experiment, it expressed little

action on the discrimination of treatment when compared to CP1.

Computerized analysis is effective for detecting lots with physiological quality differences and the results were correlated with those obtained by others vigor tests on seeds of various species, such as melon (Marcos-Filho et al., 2006) soybean (Marcos Filho et al., 2009), peanuts (Marchi et al., 2011), crotalaria (Silva et al., 2012), sweet corn (Alvarenga et al., 2012), eggplant (Silva & Cícero, 2014), and bean (Gomes Junior et al., 2014). However, this relationship was not observed for rapeseed seeds (Tohidloo & Kruse, 2009) and sunflower (Caldeira et al., 2014).

Thus, by providing reliable and reproducible results, the indexes obtained by SVIS®, associated to assessment procedures of the physiological quality of seeds, it can be included in quality control programs in the stages of production process and use of peanut seeds. Moreover, it can provide important information about the potential of initial growth and seedling emergence uniformity, which are one of the pillars for the establishment success of plants in the field.

**Table 2** - Correlation between each principal component and evaluation tests of physiological quality of peanut 'Runner IAC 886' seeds.

Variable	( <sup>1</sup> )CP1	( <sup>2</sup> )CP2
Germination	-0.95	0.22
First count	-0.95	0.23
Emergency speed index	-0.95	0.23
Accelerated aging	-0.89	0.36
Electrical conductivity	0.92	-0.30
Tetrazolium	-0.89	0.29
Seedling emergence in the field	-0.92	-0.11
SVIS <sup>®</sup> - Vigor index	-0.82	-0.57
SVIS <sup>®</sup> - Growth index	-0.81	-0.58
SVIS <sup>®</sup> - Uniformity index	-0.83	-0.55

(<sup>1</sup>) PC1: Principal component 1. (<sup>2</sup>) PC2: Principal component 2.

## Conclusions

The vigor, growth and uniformity indexes provided by automated image analysis (SVIS<sup>®</sup>) has a strong relationship with the others procedures for assessment of the physiological potential of peanut seeds from different stages of the production process.

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