

Short Research Article

Computer vision as a complementary method to vigor analysis in maize seeds**ABSTRACT**

Rapid and accurate evaluation of seed lot physiological potential are strongly desirable for success of quality control programs conducted by seed industry. This study aims to evaluate the efficiency of computer vision through a free software of processing seedling digital images, in order to characterize maize seeds physiological potential and make comparisons among routine vigor tests, recommended for this species. So that, germination test, first germination count, seedling emergence, cold test, germination speed index and electrical conductivity test were used for featuring physiological potential of maize seed lots. Then, these tests' results were compared with data collected, using image analysis technique, through SAPL[®] software. Seedlings growth were evaluated by photographs at seventh day and obtained the values of primary root, coleoptile and whole seedlings length, as well as growth, uniformity, vigor and corrected vigor indices. The computerized images analysis of seedlings through SAPL[®] is a consistent and promising alternative for evaluating the physiological potential of maize seeds. Its efficiency was proved in this study, being equivalent to what verified in routine tests for vigor determination.

Keywords: *free software; seed technology; vigor; Zea mays.*

1. INTRODUCTION

Agriculture associated to technological innovation is a key to promote qualitative and quantitative advances in food production. It is widely believed that the seed plays an important role to achieve gains in productivity. This fact is due to seed being considered a vector that frequently incorporate new technologies.

In terms of planting success, is indispensable to use seeds with a high physiological potential. That means seed with the ability to restore its biochemical system functions and result in a normal and vigorous plant [1]. Therefore, the evaluation of seed physiological quality is necessary and it is usually performed by germination test and complemented by vigor tests. There are many ways to evaluate seed vigor, such as determination of seedling growth, speed and uniformity [2,3]. However, when seedling growth parameters are manually obtained by demanding more time and may causing reading errors, mainly as a result of the analysts' subjective interpretations [4].

Given the above, the automation of those analyses through computer vision systems become a valuable alternative. It provides significant advances not only in methodology standardization by making possible to increase the analyses agility, but also in obtaining information with high reliability and repeatability level [5]. Consequently, seedlings digitized images evaluation enables lots distinguish at different vigor levels by using a fast and precise method [6,7]. Several authors report good results in an image analyses application techniques to evaluate seed quality different species, such as *Crambe abyssinica* [8], *Triticum aestivum* [9], *Zea mays* [10,11].

In order to make high-performance image analysis methods more accessible, free software and low-cost equipment may be used to acquire, process and generate image data. Seedling Analysis System (SAPL[®]) is one of the free alternatives available for this purpose [12]. Recently developed, SAPL[®] was designed to evaluate maize seed vigor, based on seedling growth. Its system has a differential in capturing images made from photographs by using cameras or smartphones. It is a quick, simple and economical acquisition that may be performed in any illuminated place.

Considering the importance of more robust methodological approaches in seed quality determination, this study aims to evaluate the efficiency of computer vision through a free software of processing

seedling digital images, in order to characterize maize seeds physiological potential and make comparisons among routine vigor tests, recommended for this species.

2. MATERIAL AND METHODS

2.1 Location and characterization of seed water content

This study was conducted at the Universidade Federal do Rio de Grande do Norte in Seed Analysis Laboratory, located in Macaíba - RN, Brazil. Four seed lots of maize varieties from the Macaíba region were used in order to evaluate their physiological quality.

First, the seed water content was determined by the oven method, at 105 ± 3 °C for 24 hours, based on The Rules for Seed Analysis [13]. So that, two replicates of 20 seeds were used for each lot.

2.2 Evaluation of seed physiological quality

The viability and vigor of maize seed lots were evaluated through the routine tests and the computer vision analysis using SAPL[®] software, described as follows:

Germination test: Four replicates of 50 seeds per lot were placed on germination paper rolls, moistened with distilled water in an amount equivalent to 2.5 times of the dry paper mass. The rolls were arranged in plastic bags and kept into a B.O.D. type germination chamber at 25°C, for seven days. Evaluations were made in accordance with the Rules for Seed Analysis [13] and the results were registered in percentage of normal seedlings per each lot.

First germination count: performed along with the germination test by counting the number of normal seedlings on the fourth day after sowing [13].

Seedling emergence: Four replicates of 50 seeds per lot were sowed in expanded polystyrene trays of multiple cells. Each cell was filled with washed sand and contained one seed that was irrigated daily and kept in a greenhouse. The emergence evaluation was performed 14 days after sowing and the results expressed as a percentage of emerged seedlings.

Cold test without soil: Four replicates of 50 seeds per lot were placed on germination paper rolls, moistened with distilled water in an amount equivalent to 2.5 times of the dry paper mass. Then, the germination paper rolls were arranged into plastic bags, sealed with adhesive tape and kept in a humid chamber at 10°C, for seven days. After that, the rolls were removed from the plastic bags and transferred to a germinator at 25°C, where remained for four days, when the number of normal seedling was counted [13].

Germination speed index: performed along with the germination test through daily counts of the normal seedlings from fourth to seventh day after sowing. GSI was calculated using equation proposed by Maguire[14].

Electrical conductivity test: four replicates of 50 seeds per lot were weighed using precision balance (0.01 g), placed into plastic cups containing 75 mL of distilled water and arranged in germination chambers at 25°C, for 24 hours. After that, the electrical conductivity of the solution was determined by using a conductivity meter (TEC-4MP model). The results were divided by the seed weight and expressed in $\mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$ [15].

Computerized image analysis: First, the maize seeds were germinated in accordance with Nakagawa et al. [2] methodology. Four replicates of 25 maize seeds per lot were longitudinally arranged in a row drawn on the upper third of germination paper. Seeds were placed in a way that the hilum was facing to the paper bottom. Then, the germination papers rolls were packed in plastic bags and placed vertically in the germinator, for seven days at 25 °C. On the seventh day, secondary roots were removed when necessary, so only primary root was left. After that, the seedlings were transferred to a blue satin vinyl foam sheet (40 x 60 cm), containing nine cells of 5 cm width and divided by white stripes. The upper corner of the first cell on the right was assigned to a scale and the rest of the cells

were individually occupied by each seedling. After recording the initial values, seedlings images were carried out by repetition and lot.

The software provided measurements of primary root, coleoptile and whole seedling length, as well as uniformity, growth, vigor and corrected vigor indices. These indices were defined by Sako et al. [7], expect for the corrected vigor index [12].

2.3 Experimental Design and Statistical Analysis

The statistical design was completely randomized with four replicates per treatments. The variance analysis was applied ($P \leq 0.01$) to data and means were compared by the Tukey test ($P \leq 0.05$). The variables mean values of vigor tests and image analysis were also correlated by the Pearson correlation coefficient (r , $P \leq 0.05$). The R software was used to statistical analyses[16].

3. RESULTS AND DISCUSSION

The seed moisture content varied from 8.5 to 9.2%, showing uniformity in the dry state maintenance, which is in accordance with Carvalho & Nakagawa[17] recommendations (8 to 10%). Marcos-Filho[18] indicates moisture content among seed lots should be low in order to compare seed physiological quality, without alters this parameter's results.

The germination test showed differences in seed viability between lots 2 and 3, with the highest and lowest normal seedlings percentage, respectively (Fig. 1A). For all lots, germination was within Brazilian commercialization standards of maize seeds, which is 85% [19], indicating high viability of the lots. Although germination test has showed similar behavior among the majority of the lots, it is possible that it may have a difference in the physiological quality among them, since the germination test is carried out under ideal conditions, such as temperature, humidity and adequate luminosity. So that, it may overestimate the real seed physiological potential by narrowing the differences among lots. According to Silva & Cicero [20], it is necessary to complement the germination test information using vigor tests that provides more sensitive indices of seed physiological quality.

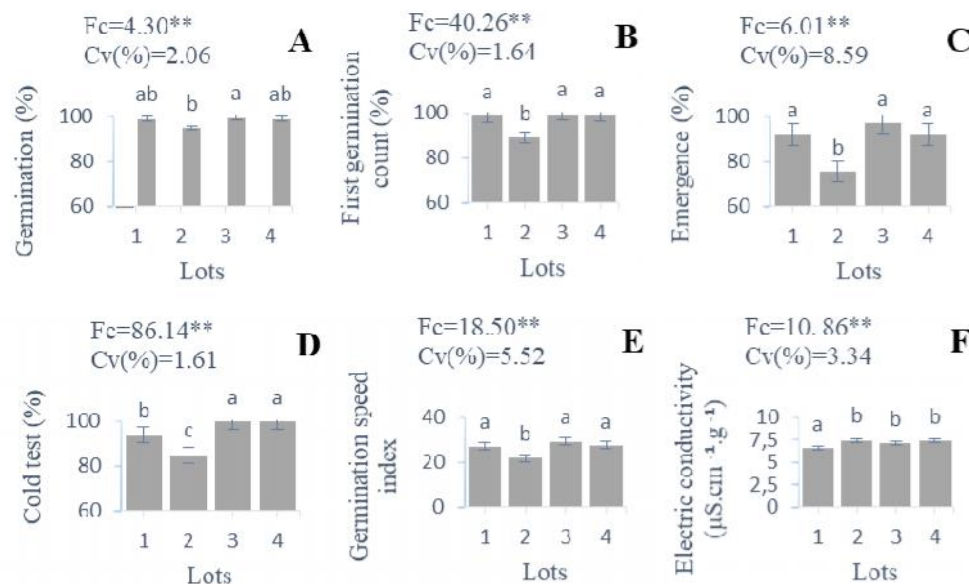


Fig. 1. Viability and vigor of four maize seed as determined through different traditional tests
^{**}Significant and ^{ns} non-significant effect by F test at 1% probability. Means followed by same later do not differ by the Tukey test t 5% probability. Fc = calculated F; CV = coefficient of variation

It was observed that lot 2 had a lower performance on the first germination count, the seedling emergence and the germination speed index (Fig. 1B, 1C, 1E). All tests ranked lots into two vigor classes as occurred in the germination test. According to Pessarakli [21], the seedling emergence duration express the speed, in which metabolic systems and embryo structures are recovered. So,

high values of this parameter are related to high physiological quality as may be observed to majority of studied lots.

In the cold test (Fig. 1D), high germination rates were reached. However, this test was capable to differentiate lots into vigor classes, so that lots 3 and 4 had high vigor, lot 1 and 3 had intermediate and low vigor, respectively. The cold test is the most used test to evaluate maize seeds, especially in low temperature areas during sowing [22]. In this study, the cold test was the most sensitive to characterize maize seeds lots, being complementary to germination test. This test efficiency is due to vigorous seeds have a higher probability to survive under low temperature. This fact is related to the difficult of cellular membrane reorganization during imbibition in those conditions [23].

By the electrical conductivity test (Fig. 1F), it was possible to identify the difference in vigor between lot 1 and the others, being this lot considered as having a higher physiological potential, showing less exudates leaching. Otherwise in the cold test, the electrical conductivity test ranked lots into less vigor classes and classified lots into different performances. Pinto et al.[24] alleges that tendency is normal since those tests evaluate different seed characteristics.

Evaluating seed vigor through seedling image analysis on seventh day after the germination test begun enabled to identify differences among physiological potential of the lots by using most of the SAPL[®] provided variables (Fig. 2).

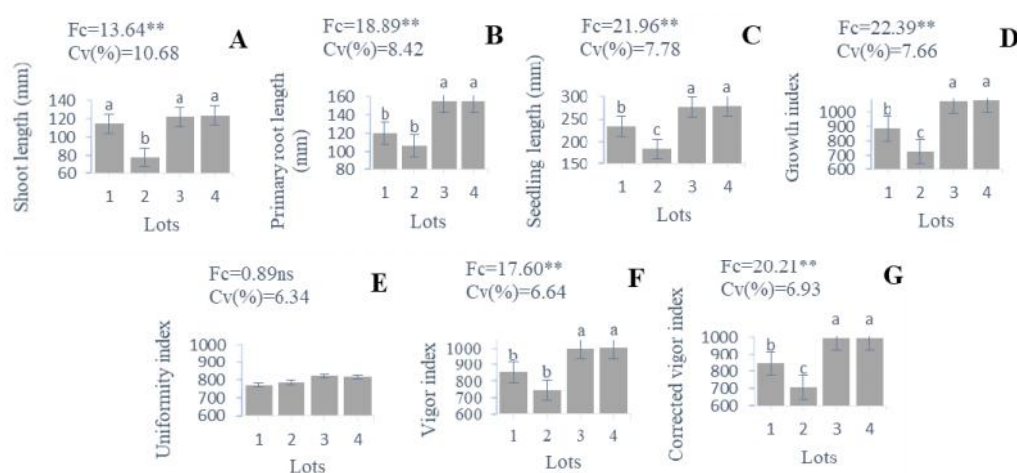


Fig. 2. vigor of four maize seed as determined image analysis through SAPL[®] software
****Significant and ^{ns} non-significant effect by F test at 1% probability. Means followed by same later do not differ by Tukey test at 5% probability. Fc = calculated F; CV = coefficient of variation**

Shoot length (Fig. 2A) enables to identify a lower performance of lot 2 compared to the other lots. Whereas, primary root length (Fig. 2B) distinguished lots 3 and 4 as high vigor and lots 1 and 2 as low vigor. Seedling length (Fig 2C) ranked lots into three classes, similar to the cold test. Those results corroborate the majority of routine test performed in this study. Other authors, such as Dias et al.[25] verified that these variables especially root lengths originated by image analysis were efficient to rank maize seed lots lead to reliable data compared to traditional vigor tests as may be shown in this study results.

Analyzing other variables provided by the SAPL[®] software, it was observed that the growth index (Fig. 2D) also enables lots classification as similar to the cold test results. It demonstrates that lots 3 and 4 had the best performance compared to lots 1 and 2. According to Rocha et al.[26], low physiological potential seeds usually have a delay in cell membrane system restructuring. This fact impacts on metabolic system causing effects on mitochondrial efficiency through lower energy release and slower seedling growth.

The uniformity index (Fig. 2E) was not an efficient variable to evaluate vigor in four studied maize seed lots, so it was not possible to identify significant differences for a categorization among the lots. Caldeira et al.[27] found similar results when evaluate sunflower seeds related to the uniformity

variable. According to Marcos Filho et al.[6], uniformity is a characteristic normally present in vigorous lots that don't contribute for distinguishing significantly the lots. So that, it is necessary to use other more sensitive parameters.

The vigor index (Fig. 2F) results shows that was possible to distinguish lot 2 from the others, being lot 2 the one with the lowest performance. Lots that reached high vigor in this test show seedlings with the highest growth rates. So that, Dan et al.[28] indicates that the higher rates may be provided by the greater tissues transformation capacity. In other words, the rapid degradation of storage tissues reservations that are used for composition and formation of embryonic axis.

The corrected vigor index (Fig. 2G) provided similar results to those observed in the initial physiological characterization, showing that it may be considered sensible for distinguishing lots, once had ranking three vigor levels: lots 3 and 4 (high vigor), lot 1 (medium vigor) and lot 3 (low vigor). The corrected vigor index takes into account not only seedling length, but also seed germination, being more coherent between viability and maximum expected vigor [12]. For Nakagawa et al.[2], this adjustment is important because in some cases the lot may have low germination percentage and high average seedling length, or the opposite. In this situation, a few normal seedlings that expected to have a low viability, presented high growth rate. So that, this fact may not be transposed to the whole sample or lot, considering it vigorous.

From the Pearson correlation analysis (Fig. 3), it was possible to observe significant correlations between variables generated by traditional tests and image analysis. Only the uniformity index didn't correlate significantly with any traditional test. Brunes et al.[9] in their research with wheat seeds, also observed a significant correlation between most of variables related to length and measured by image analysis, with the other routine tests used.

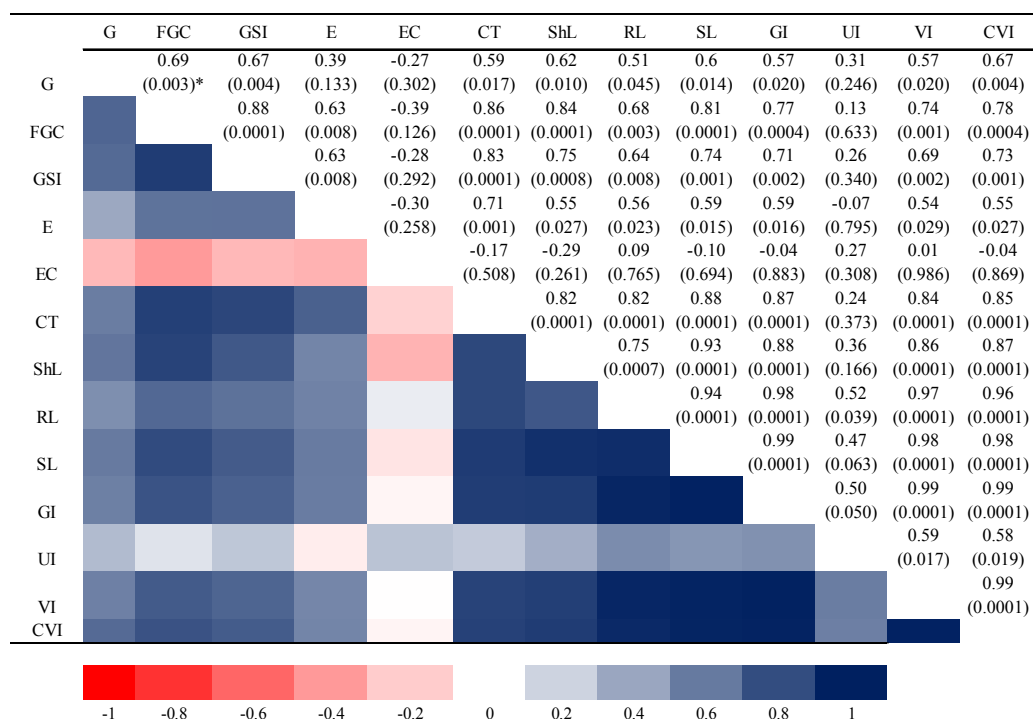


Fig. 3. Pearson correlation between variables evaluated through routine test and image analysis in seed and seedling for four lots of maize varieties

* p-value; G = germination, FGC = first germination count, GSI= germination speed index, E = emergence, CT = cold test, EC = electric conductivity, ShL = shoot length, RL = root length, SL = seedling length, GI =growth index, UI = uniformity index, VI = vigor index, CVI = corrected vigor index

The significant correlations show that the cold test, considered the most sensitive test for initial characterization, was strongly correlated with all indices generated through image analysis, except for the uniformity index. Although, the significant correlation indicates only a similar trend in variation

between two characteristics [29]. According to Albuquerque et al.[30], when there is a great dependence between the variables, may decide to reduce the number of characteristics evaluated in future experiments.

4. CONCLUSION

The computerized images analysis of seedlings on seventh day, using SAPL[®], is a consistent and promising alternative for evaluating the physiological potential of maize seeds, with proven efficiency in this study, being equivalent to what verified in traditional tests for vigor determination.

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