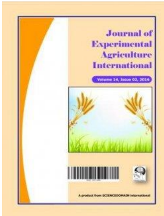

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Computer Vision as a Complementary Method to Vigour Analysis in Maize Seeds

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Authors' contributions

This work was carried out in collaboration with all authors. Authors ADM and MDP participated in all research phases, data collection, analysis and data interpretation and manuscript writing. Author TFSNS contributed in the translation manuscript for English language and in manuscript writing. Authors BGN and DTP contributed to the data analysis and manuscript writing. All authors read and approved the final manuscript.

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ABSTRACT

Rapid and accurate evaluation of seed lot physiological potential is strongly desirable for the 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 characterise maize seeds physiological potential and make comparisons among routine vigour 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 the physiological potential of maize seed lots. Then, these tests' results were compared with data collected, using an image analysis technique, through SAPL® software. Seedlings growth were evaluated by photographs on the seventh day and obtained the values of the primary root, coleoptile, and whole seedlings length, as well as growth, uniformity, vigour

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and corrected vigour indices. The computerised 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 vigour determination.

Keywords: Free software; seed technology; vigour; Zea mays.

1. INTRODUCTION

Agriculture associated with 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 in achieving gains in productivity. This fact is due to seed being considered a vector that frequently incorporates new technologies.

In terms of planting success, it is indispensable to use seeds with a high physiological potential. That means seeds with the ability to restore its biochemical system functions and result in a normal and vigorous plant [1]. Therefore, the seed physiological quality evaluation is necessary, and it is usually performed by germination test and complemented by vigour tests. There are many ways to evaluate seed vigour, such as determination of seedling growth, speed and uniformity [2,3]. However, when seedling growth variables are measured manually using ruler or calliper, the evaluations become time-consuming and may lead to errors in reading, mainly due to the subjective interpretations of the analysts [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 standardisation by making possible to increase the analyses agility, but also in obtaining information with high reliability and repeatability level [5]. Consequently, seedlings digitised images evaluation enables lots to distinguish at different vigour 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* Leão-araújo et al. [8], *Triticum aestivum* Brunes et al. [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 vigour, based on seedling growth. The software has 97% accuracy and takes an average of 0.19 seconds to analyse each seedling. Its system has a differential in capturing images made from photographs by using cameras or smartphones. It is a quick, economical and straightforward acquisition that can 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, to characterise maize seeds physiological potential and make comparisons among routine vigour tests, recommended for this species.

2. MATERIALS AND METHODS

2.1 Location and Characterisation 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 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 vigour 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 the 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 seedlings was counted [13].

Germination speed index: performed along with the germination test through daily counts of the normal seedlings from the fourth to the seventh day after sowing. The germination speed index was calculated using the 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 $\mu S.\ cm^{-1}\ g^{-1}$ [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 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 the primary root, coleoptile and whole seedling length, as well as uniformity, growth, vigour and corrected vigour indices. These indices were defined by Sako et al. [7], except for the corrected vigour index [12].

2.3 Experimental Design and Statistical Analysis

The statistical design was completely randomised 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 vigour tests and image analysis were also correlated by the Pearson correlation coefficient *(r, $P \leq 0.05$).* The R software was used for 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 and Nakagawa [17] recommendations (8 to 10%). Marcos-Filho [18] indicates moisture content among seed lots should be low to compare seed physiological quality, without alters this parameter's results.

The germination test showed differences by the Tukey test 5% probability of 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 commercialisation standards of maize seeds, which is 85% [19], indicating the high viability of the lots. Although germination test has shown similar behaviour 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. Therefore, 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 vigour tests that provide more sensitive physiological quality seed indices.

It was observed that lot 2 had lower performance in all initial characterisation tests, except in the electrical conductivity test (Fig. 1). All tests ranked lots into two vigour classes as occurred in the germination test. According to Pessarakli [21], the seedling emergence duration expresses the speed, in which metabolic systems and embryo structures are recovered. So, high values of this parameter are related to high physiological quality as observed in the majority of studied lots.

In the cold test (Fig. 1D), the germination rate of the less vigorous lots was observed. Thus, this test was able to differentiate the lots into vigour classes, so that lots 3 and 4 had high vigour, lots 1 and 2 had intermediate and low vigour, 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 characterise maize seeds lots, being complementary to the 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 reorganisation during imbibition in those conditions [23].

By the electrical conductivity test (Fig. 1F), it was possible to identify that lot 1 presented lower leaching of exudates, differing statistically from the other lots. Electrical conductivity test ranked seed lots into a lower vigour level compared to the cold test, according to Pinto et al. [24] this is normal since the tests evaluate the different seed characteristics.

Evaluating seed vigour through seedling image analysis on the 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).

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 vigour and lots 1 and 2 as low vigour. 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 vigour 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 restructuration. This fact impacts on the 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 vigour in four studied maize seed lots, so it was not possible to identify significant differences for a categorisation among the lots. Caldeira et al. [27] found similar results when evaluating 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 the lots significantly. So, it is necessary to use other more sensitive parameters.

The vigour index (Fig. 2F) results show that it was possible to distinguish the lots at two levels of vigour, in which lots 1 and 2 presented lower performance. Lots that reached high vigour in this test show seedlings with the highest growth rates. According to Dan et al. [28], these higher rates were provided by the greater capacity degradation of storage tissue stores for use in embryonic axis composition and formation.

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 vigour), lot 1 (medium vigour) and lot 2 (low vigour). The corrected vigour index takes into account not only seedling length, but also seed germination, being more coherent between viability and maximum expected vigour [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 were 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 (without image analysis). Brunes et al. [9] in their research with wheat seeds, also observed a significant correlation between most of the variables related to length and measured by image analysis, with the other routine tests used.

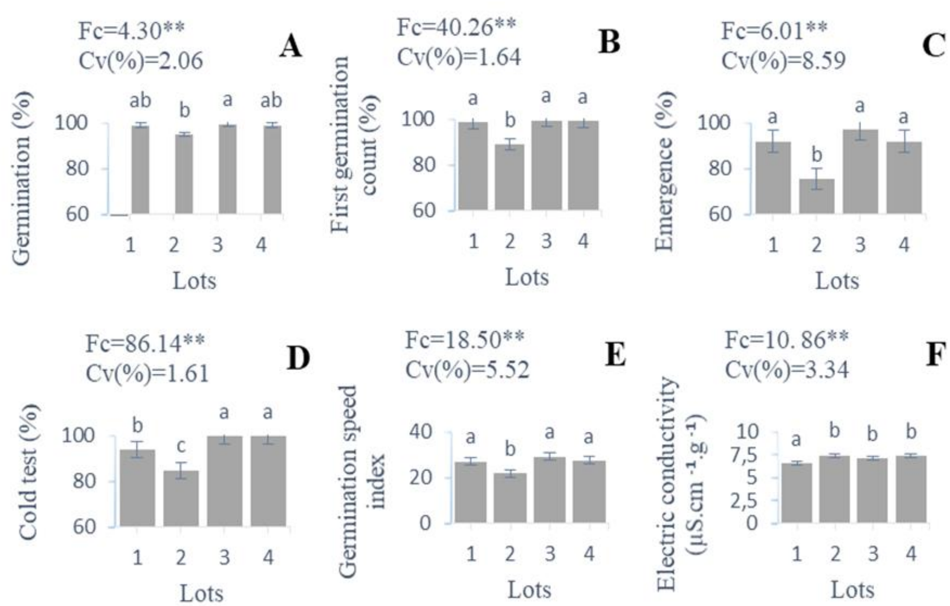

Fig. 1. Viability and vigour of four maize seed as determined through different traditional tests
***Significant and ^{ns} non-significant effect by F test at 1% probability. Means followed by same later do not differ by the Tukey test 5% probability. Fc = calculated F; CV = coefficient of variation*

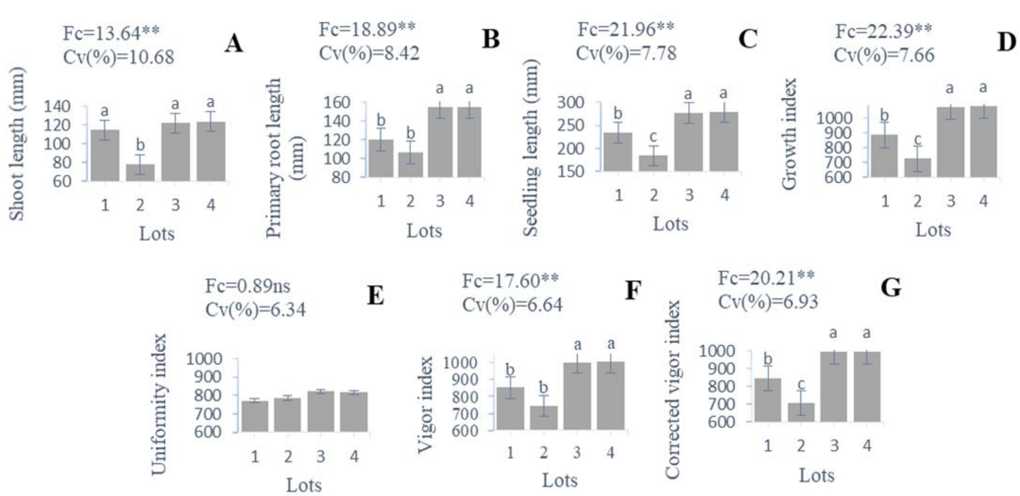

Fig. 2. Vigour of four maize seed as determined by image analysis through SAPL® software
***Significant and ^{ns} non-significant effect by F test at 1% probability. Means followed by same later do not differ by Tukey test at 5% probability. Fc = calculated F; CV = coefficient of variation*

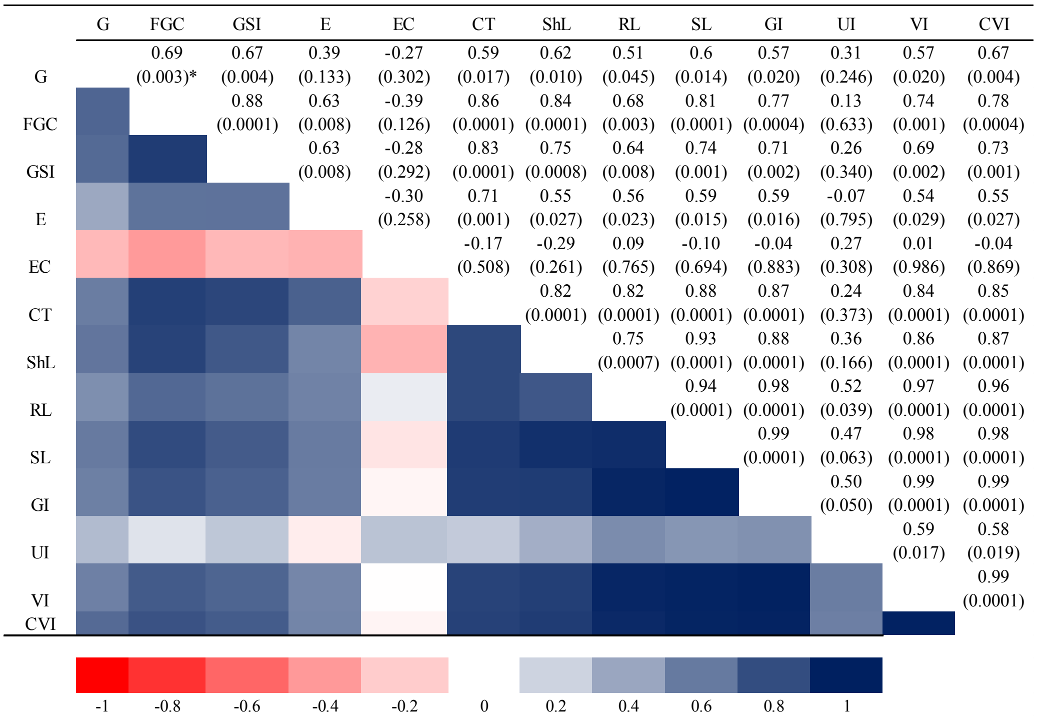

Fig. 3. Pearson correlation between variables evaluated through routine test and image analysis in seed and seedling for four lots of maize varieties

** p-valor; 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 characterisation, 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 an excellent dependence between the variables, may decide to reduce the number of characteristics evaluated in future experiments.

4. CONCLUSION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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