Recent approaches for bell pepper seed vigor testing

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ABSTRACT. Seed vigor is a major attribute in the determination of whether a seed lot can be commercialized. Different physiological tests are used to assess seed vigor and are generally based on different concepts, which could lead to variable interpretations, particularly when comparing lots presenting different vigor levels. This study was conducted to compare the different approaches used to evaluate bell pepper seed vigor and verify the applicability of the Seed Vigor Imaging System (SVIS) for this purpose. The experiment was performed using four seed lots of bell pepper hybrids in two periods, and the evaluations involved germination, seedling emergence, seedling emergence speed index, saturated salt accelerated aging and image analysis. The use of seedling imaging analysis by SVIS is a new approach for bell pepper seed vigor evaluation. Both the software and traditional tests provided sufficient information for seed vigor analysis, enabling the segregation of the analyzed seed lots. Thus, the new method fulfills the requirement for an efficient, rapid, cost-effective and standardized method that allows comparisons among laboratories and avoids human influence on the interpretation of the results. SVIS can be considered a feasible option to be included in quality assurance programs of the bell pepper seed industry.

Keywords: seed analysis, SVIS, image analysis, seed quality.

Novas abordagens na avaliação do vigor de sementes de pimentão

RESUMO. Para a avaliação do vigor de sementes podem ser utilizados uma série de testes fisiológicos, os quais, baseados em diferentes conceitos, podem resultar em diferentes interpretações dos resultados obtidos, especialmente quando há comparações de lotes com diferentes níveis de vigor. O objetivo desse trabalho foi comparar diferentes abordagens para avaliação do vigor de sementes de pimentão e verificação da aplicabilidade do programa computacional 'Seed Vigour Imaging System' (SVIS) para este propósito. Dessa forma, em dois períodos de avaliação, avaliou-se quatro lotes de sementes de pimentão por meio dos testes de germinação, emergência de plântulas, envelhecimento acelerado e análise de imagens. O uso do SVIS pode ser considerado uma nova abordagem para avaliação do vigor de sementes de pimentão. Tanto o programa computacional como os testes tradicionais forneceram informação suficiente para a análise de vigor de sementes, permitindo igualmente a segregação dos lotes analisados e, ainda, preenchem requisitos importantes para um teste laboratorial, como padronização, eficiência, rapidez e baixo-custo. Assim, permitem a comparação entre laboratórios e diminuem os riscos da influência humana na interpretação de resultados, podendo ser considerado uma possível opção para ser incluída em programas de controle de qualidade na produção de sementes de pimentão.

Palavras-chave: análise de sementes, SVIS, análise de imagens, qualidade de sementes.

Introduction

In horticultural crops, such as bell pepper (*Capsicum annuum* L.), seeds are generally sown in multicellular trays for bedding plant production before transplantation to the field. Consequently, because of the high cost of seeds, there is a strong demand for seed lots with a high percentage of germination and rapid and uniform seedlings growth. Seed vigor affects the seed viability, speed of germination, seedling growth, seedling sensitivity to external factors and seed lot storability (CORBINEAU, 2012). Thus, seed companies rely on vigor tests to assure the high quality of seed lots for bedding plant growers.

Seed vigor tests are based on different concepts, such as the resistance to stress, speed of germination, membrane integrity and seedling development (MATTHEWS et al., 2012). As based on the recently available technologies and physiological discoveries, the evaluation methods are constantly improving (KODDE et al., 2012; MATTHEWS et al., 2012), however the variety of models can result in different interpretations of seed vigor.

Despite the reliability of available seed vigor tests, there are still opportunities to improve or develop new methods (MARCOS FILHO et al., 2009), and there is considerable interest in developing methods and equipment that enable a rapid and automated evaluation of seed quality. The Seed Vigor Imaging System (SVIS) was developed at the Ohio State University and has been proposed as an alternative to traditional vigor tests (GOMES JR. et al., 2009; HOFFMASTER et al., 2003; HOFFMASTER et al., 2005; MONDO et al., 2011; SILVA et al., 2012; MARCOS FILHO; KIKUTI, 2014). The software enables the analysis of seedling images, providing indexes of growth, uniformity and vigor, a rapid and objective measurement of seed quality (PEÑALOZA et al., 2005) and avoids human error during the evaluations (HOFFMASTER et al., 2003; HOFFMASTER et al., 2005). Computerized image analysis has been reported to be an accurate method to measure also seedling growth rate for different species (DELL'AQUILA, 2007).

This study was conducted to compare the different approaches used for bell pepper seed vigor assessment and the applicability of SVIS for seed vigor testing in this crop.

Material and methods

The experiment was conducted in the laboratories of Seed Analysis and Image Analysis, in the Crop Science Department of the College of Agriculture 'Luiz de Queiroz', University of São Paulo, in Piracicaba, São Paulo State, Brazil, from 2009 to 2011.

Plant material

The experiment was conducted using four seed lots of the bell pepper hybrid 'Magali AF-249', with each lot being produced in different production seasons and locations. The plant material was provided by Sakata Seed Sudamerica, in Bragança Paulista, São Paulo State, Brazil.

Physiological tests

Two evaluations were conducted twenty months apart. Soon after the first evaluation, the seed lots were stored under controlled conditions of temperature (20°C) and air humidity (45%). The physiological tests conducted for both periods are described below.

Moisture content: determined by oven drying two seed samples of 2-g from each seed lot at 105°C ± 3°C for 24 hours. Thereafter, the samples were weighted, and the seed moisture was expressed as a percentage (BRASIL, 2009).

Germination (G): conducted with four replications of 50 seeds per lot in plastic boxes (11.5 x 11.5 x 3.5 cm) containing two sheets of blotting paper watered with 2.5 times its dry weight. These boxes were placed in germination chambers regulated at 25°C. The germination assessment occurred on the seventh and fourteenth days after sowing, and the results were expressed as the percentage of normal seedlings (BRASIL, 2009).

Seedling emergence (SE): four replications of 50 seeds per lot were randomly distributed in polystyrene trays with individualized cells (one seed per cell) filled with commercial substrate (Plantmax – Hortaliças). These trays were placed in a greenhouse at a non-controlled temperature and watered as needed. The emerged seedlings were counted daily to calculate the seedling emergence speed index (SESI) (MAGUIRE, 1962). The final evaluation occurred on the fourteenth day, and the results were expressed as a percentage.

Saturated salt accelerated aging (SSAA): the procedure proposed by Jianhua and McDonald (1997) for small seeds was used as the main comparison test. The test was conducted with 2-g samples from each seed lot placed as a single layer on a screen in plastic boxes (11.5 x 11.5 x 3.5 cm) containing 40 mL of saturated solution of sodium chloride (deionized water with 16 g of dissolved NaCl). These plastic boxes were then placed in chambers at 41°C for 48 hours. Using four replications of 50 seeds, a germination test was then conducted, as described above.

Seed vigor imaging system (SVIS): conducted with four replications of 25 seeds per lot following the method described above for the germination test. The plastic boxes were positioned in the germination chamber at an inclination of 45° to promote straight root growth. On the sixth day after sowing, the seedling images were captured using an inverted scanner (HP Scanjet 2004), and the images were processed by the software Photosmart with a resolution of 100 dpi. These images were then analyzed by the software Seed Vigor Imaging System (SVIS), which computed a growth index and a uniformity index, both ranging from 0 (no germination) to 1000 (maximum germination). The software provided an overall vigor index (VI), which, based on works of Gomes Jr. et al. (2009), Marcos Filho et al. (2009), Kikuti and Marcos Filho (2012), Silva et al. (2012) and Chiquito et al. (2012), consisted of the sum of 70% of the growth index (GI) and 30% of the uniformity index (UI).

Statistical analysis

The experiment was conducted in a completely randomized design with four replications. The data were subjected to an analysis of variance, and the means were compared by the Tukey test at a 5% level of significance.

Results and discussion

The average moisture content of the seed lots are presented in Table 1. Acceptable differences among the aged seed lots for both periods were found using the saturated salt accelerated aging test (SSAA), varying less than two percentage points, which is considered the maximum variation for this test (MARCOS FILHO, 2005), and guaranteeing confidence in the results. It is interesting that seed lot four, the driest seed lot, was identified as the least vigorous, indicating that the differences observed before and after the physiological tests did not impact the results (PANOBIANCO; MARCOS FILHO, 1998).

Table 1. Initial moisture contents (MC; %) and after salt saturated accelerated aging test for seed lots of hybrid Magali AF-249 hybrid in both evaluation periods.

Seed Lots	Before storage		After storage	
	Initial seeds MC	Aged seeds MC	Initial seeds MC	Aged seeds MC
Lot 1	7.7	11.0	6.4	9.9
Lot 2	7.2	10.5	6.6	10.3
Lot 3	6.4	10.8	6.1	10.7
Lot 4	5.2	10.1	6.8	9.0

In the first period of evaluation (Table 2), lot one was considered to be a high-vigor seed lot, presenting the highest values for the seedling emergence speed index (SESI) and SSAA, with lots three and four, low-vigor lots, presenting the lowest values for the same evaluations. In addition, based on the seedling emergence (SE) results, it was possible to classify lots one and two as high performance, lot three as intermediate performance and lot four as low performance, results similar to those found using the germination assay.

Table 2. Physiological tests for seed lots of hybrid Magali AF-249 before the storage period.

Seed lot	G (%)	SE (%)	SESI	SSAA (%)	VI	GI	UI
Lot 1	96a[*]	95a	34.9a	92a	273a	300a	214a
Lot 2	94a	93a	27.9b	80b	206a	217b	181a
Lot 3	85b	62b	12.1c	56c	127b	106c	178a
Lot 4	72c	48c	9.5c	52c	66b	68c	65b
CV (%)	3.6	7.5	7.7	5.7	18.7	21.5	21.1

[*]In columns, means followed by same letter are equal by Tukey test (5%). G – Germination; SE – Seedling Emergence; SESI – Seedling Emergence Speed Index; SSAA – Salt Saturated Accelerated Aging; VI – SVIS Vigour Index; GI – SVIS Growth Index; UI – SVIS Uniformity Index.

By analyzing the SVIS data, the indexes of vigor (VI) and uniformity (UI) indicated a higher quality for lots one and two, as did the seedling emergence test. Contreras and Barros (2005) utilized SVIS for lettuce seed vigor analysis and also showed a high correlation with seedling emergence in a greenhouse, in addition to the other vigor tests examined. Marcos Filho et al. (2006) concluded that the SVIS results for ten seed lots of two melon cultivars correlated better with the seedling emergence and speed of emergence at 12 and 18°C compared to other tests of vigor. The results obtained in the present work agreed with those of Marcos Filho et al. (2006), showing that SE, VI and UI well segregated the seed lots. Additionally, the growth index (GI) values showed similarity to the SESI and SSAA results, clearly identifying lot one as having the highest quality seeds, whereas lots three and four had the poorest quality seeds.

After 20 months of storage (Table 3), the seed lots continued to present the same pattern of seed quality differences. With regard to germination, lots one, two and three had the same performance after storage and were classified as the highest quality lots. For SE, SESI and SSAA, lot one remained the highest quality seed lot and lot four the lowest quality. Lots two and three showed variable performance for those tests and were classified as having intermediate seed vigor. Those results indicate a different response of the seed lots to storage, which is also known as storability (CORBINEAU, 2012). Additionally, SESI offered a more detailed seed vigor analysis than SE.

Table 3. Physiological tests for seed lots of hybrid Magali AF-249 after the storage period.

Seed lot	G (%)	SE (%)	SESI	SSAA (%)	VI	GI	UI
Lot 1	97a[*]	93a	26.0a	91a	110a	39a	277a
Lot 2	94a	67b	13.5b	74b	122a	37a	321a
Lot 3	89a	84b	8.4c	49c	70b	25b	175a
Lot 4	72b	54c	4.7c	39c	25c	7c	68b
CV (%)	4.8	16.4	20.4	16.0	17.3	25.4	17.5

[*]In columns, means followed by same letter are equal by Tukey test (5%). G – Germination; SE – Seedling Emergence; SESI – Seedling Emergence Speed Index; SSAA – Salt Saturated Accelerated Aging; VI – SVIS Vigour Index; GI – SVIS Growth Index; UI – SVIS Uniformity Index.

The differences between lots one and two occurred to a lesser extent during the second period of evaluation (Table 3), showing equality for five of the seven tests, whereas they were equal in four of the seven tests in the first period. Seed vigor testing has been mainly used to detect differences related to seed lot performance during storage or after sowing, with the aim of identifying seed lots that will be able to establish a proper crop development under various environmental conditions (MARCOS FILHO et al., 2009).

Moreover, considering the accuracy of the SE, SESI and SSAA tests in differentiating the bell

pepper seed lots by vigor, these tests were considered suitable for comparisons with the SVIS results. The VI, as assessed using 6-day-old seedlings by SVIS (Table 2), presented higher values for lots one and two and a detailed segregation among lots one, two, three and four. The UI indicated lots one and two as the most uniform in seedling development. The results of GI were similar to the results of SSAA, enabling an accurate segregation of the high (lot one and two), intermediate (lot three) and low (lot four) quality lots.

A remarkable advantage of the SVIS method is the potential of representing seedling growth and uniformity in the same index (VI), thus making it a more informative method than other vigor tests. Principally, it was possible to observe that the UI showed similarity between lots one and two before storage, whereas similarity was only identified after storage according to the GI evaluation.

The results from the SVIS analysis presented higher coefficients of variation than the SE, SESI and SSAA results. These findings can be explained to a certain extent by the parameter used in this test, which is based on seedling length and, therefore, produces more variable data than the percentage of germination or seedling emergence. The determination of the seed physiological potential by the comparison of the seedling length or one of its parts is a procedure with proven efficiency (STEINER, 1990). Additionally, SVIS could be considered a direct vigor test due to its measurement of the overall performance of seedlings, regardless of the factors compromising seed performance, including disease or mechanical damage. Higher coefficients of variation could mask small differences of seed vigor among seed lots. However, the detailed analysis as an overall performance provided by SVIS enabled an appropriate vigor characterization of the seed lots, emphasizing the high quality of this test. Furthermore, the coefficient of variation increased for SE, SESI and SSAA in the second period of evaluation, whereas the coefficients for SVIS were considered to be similar in the first period. This test offers valuable information about the initial growth potential and uniformity of seedling emergence, which are very important for modern agriculture, particularly for horticultural crops.

The SVIS software has been applied to seed vigor evaluations in various species, such as corn (HOFFMASTER et al., 2005), sweet corn (GOMES JR. et al., 2009), soybean (HOFFMASTER et al., 2003; MARCOS FILHO et al., 2009), lettuce (KIKUTI; MARCOS FILHO, 2012; PEÑALOZA et al., 2005), sun hemp (SILVA et al., 2012),

cucumber (CHIQUITO et al., 2012) and melon (MARCOS FILHO et al., 2006). The software has also been recommended as a useful and reliable alternative test for seed vigor assessment in comparison to traditional vigor tests. Hoffmaster et al. (2003) and Marcos Filho et al. (2009) demonstrated the potential of this software in evaluating the vigor of soybean seeds using 3-day-old seedlings, and Mondo et al. (2011) showed the applicability of this system when the evaluation period was reduced from three to two days for corn seedlings. These studies and the results obtained in this experiment indicate that SVIS is a very useful technique due to its capacity to offer rapid, reliable and reproducible results, mainly through standardization, as the results are computer acquired using the same software from location to location, a significant advantage over vigor tests interpreted by humans.

Therefore, the SVIS evaluation of bell pepper seed lots can be easily and accurately practiced in laboratories at a relatively low cost. Additionally, the results are not subject to the influence of human interpretations and could reduce the time and cost spent on seed analyses. Furthermore, it is always important to evaluate the physiological potential of seed lots using at least two different tests and to perform a careful interpretation of the results to obtain a high consistency in generating reliable information (CHIQUITO et al., 2012).

Conclusion

Seedling imaging analysis using the Seed Vigor Imaging System (SVIS) is a new approach for bell pepper seed vigor testing and showed sufficient sensitivity for potential physiological assessment compared to the results provided by traditional vigor tests. This method fulfills the requirement for an efficient, rapid, cost-effective and standardized method that allows comparisons among laboratories and avoids the influence of human interpretations of the results.

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