



Seedling performance-based methodologies for assessing melon seed physiological quality

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Abstract

While numerous methods exist for assessing seed physiological quality, seedling performance-based approaches often lack species-specific adaptations. This study evaluated the efficacy of various seedling performance methodologies for assessing physiological quality in yellow Valenciano-type melon (*Cucumis melo* L.) seed lots. Four seed lots underwent comprehensive testing, including: water content, germination, first count, electrical conductivity, root dry mass, hypocotyl/primary root length, greenhouse emergence, and multiple germination/emergence velocity indices (original and corrected forms). Strong correlations emerged between velocity indices and laboratory tests, though these relationships disappeared under field conditions. Our findings demonstrate that seedling-based assessments effectively distinguish melon seed vigor in controlled laboratory environments, but lose predictive validity in field applications due to environmental interactions. These results highlight the need for species-specific vigor protocols and caution against extrapolating laboratory results to field performance predictions.

Keywords: *Cucumis melo* L.; velocity of germination; seed vigor

Introduction

Melon (*Cucumis melo* L., Cucurbitaceae) is a globally agronomically significant species widely cultivated due to its commercial value and edaphoclimatic adaptability. In Brazil, the melon production chain has become a paradigm of accelerated modernization, marked by the systematic adoption of technological innovations, increased production efficiency, and significant socioeconomic impacts—particularly in the semi-arid region, where it serves as a key driver of employment and income generation.

The sustained expansion of this crop has required continuous genetic improvement programs aimed at developing genotypes with resistance to biotic and abiotic stresses, as well as superior qualitative traits, alongside optimized production technologies and strict seed quality control protocols. To meet national demand for vegetable production with high yield and

agronomic uniformity, the use of seeds with proven high physiological quality is essential. Even when commercial seed lots exhibit high germination rates under controlled conditions, seedling performance in the field may vary due to seed vigor heterogeneity (Barbosa et al., 2021).

Differences in seed lot quality can be identified through vigor tests, including stress-based tests, biochemical tests, and seedling development-based tests. The latter offers the advantage of being conducted concurrently with germination and seedling emergence tests, enabling faster results (Ebone, 2019).

Among the most widely used methods for evaluating the physiological quality of melon seeds are electrical conductivity (Torres & Marcos-Filho, 2005), accelerated aging (Torres et al., 2009), and, more recently, ethanol release (Ornellas et al., 2020). Although effective, these tests require specific laboratory protocols, time, and resources. In contrast, seedling performance-

based methods, such as vigor classification and various germination speed indices, can provide critical insights using only germination and seedling emergence tests (Matthews et al., 2012), as demonstrated for pumpkin seeds (Souza et al., 2013).

Growth speed tests based on germination and primary root protrusion (≥ 2 mm) have been validated for watermelon, melon, and cucumber seeds (Mavi et al., 2010), soybean (Rego et al., 2023), and wheat (Favoretto et al., 2024). However, research on melon seeds remains limited regarding mathematical formulas and coefficients, justifying further efforts to establish standardized protocols for the execution and interpretation of seedling performance-based tests.

Given the relevance of this topic, this study aimed to evaluate the efficiency of different seedling performance-based methodologies for assessing the physiological quality of yellow melon seed lots.

Material and methods

The experiment was conducted at the Phytotechny Laboratory and in a greenhouse, both located at the State University of Santa Cruz (UESC) in the municipality of Ilhéus, Bahia, Brazil (14°47'20" S, 39°02'58" W, 52 m altitude), where the average regional temperature is 24 °C.

Four commercial seed lots of yellow Valenciano-type melon, chemically treated with the fungicide thiram (500 g L⁻¹ a.i.), were used.

The seed lots were subjected to the following tests to evaluate their physical and physiological quality:

Water content was determined by the oven method at 105°C for 24 hours (Brasil, 2009). Two replicates were used, with measurements taken after 24 hours, and results expressed as percentage (wet basis) for each lot.

The germination test was conducted with four replicates of 50 seeds per lot, distributed in paper rolls previously moistened with distilled water at 2.5 times the weight of the dry paper. Samples were germinated at 25 °C. Evaluations were performed at 4 and 8 days after sowing (Brasil, 2009), with results expressed as percentage of normal seedlings.

The first count was carried out simultaneously with the germination test, recording the percentage of normal seedlings at 4 days after sowing.

The electrical conductivity test was performed with four replicates of 50 pure fraction seeds, weighed on a precision balance (0.0001 g). The seeds were then soaked in 200 mL cups containing 75 mL of deionized water for 24 h at 25 °C. Following the imbibition period, the solution's electrical conductivity was measured using

a conductivity meter, with results expressed in $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$.

Seedling emergence test in greenhouse conditions was conducted with four replicates of 25 seeds per lot. Seeds were sown in polystyrene trays containing commercial Carolina Padrão substrate. Daily irrigation maintained moisture at approximately 60% of the substrate's water retention capacity. Mean temperatures naturally fluctuated within ranges of 30±2 °C (day) and 20±2 °C (night) during the experimental period. Evaluation occurred eight days after sowing, considering seedlings with primary leaves whose edges were not touching, with results expressed as percentage.

Root dry mass was determined at the end of the germination test using ten seedlings per replicate per lot. Roots were excised from shoots with a blade, placed in paper bags, and dried in a forced-air circulation oven at 65 °C until constant mass was achieved. Samples were weighed on an analytical balance, with results expressed in grams.

Hypocotyl and root length measurements were obtained at germination test completion, following the same protocol as dry mass evaluation. Ten subsamples per replicate per lot were measured using a digital caliper, with results expressed in millimeters.

Germination speed parameters were calculated from daily germination test data. Germination velocity indices were determined using the formulas proposed by Kotowski (1926) and Maguire (1962), as described by Santana and Ranal (2004). Specifically, germination speed (GS), germination velocity index (GVI), and germination speed coefficient (GSC) were calculated using the following formulas:

Germination Speed Index (Maguire, 1962):

$$GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n} \quad (1)$$

Where, G_n , number of seeds germinated on the n^{th} day and N , number of days after sowing.

Corrected Germination Speed Index (based on total germinated seeds)- GSI_c :

$$GSI_c = \frac{GSI}{G_g} \quad (2)$$

Where, GSI, original Maguire index (above) and G_g , total germinated seeds.

Germination Speed Index Corrected by Total Tested S

$$GSI_{c2} = \frac{GSI}{G_t} \quad (3)$$

Where, GSI, original Germination Speed Index (Maguire, 1962), and G_t , total number of seeds tested.

Germination Velocity Coefficient (GVC) - Kotowski

$$GVC = \left(\frac{\sum f_i}{\sum f_i x_i} \right) \times 100 \quad (4)$$

This coefficient represents the inverse of the weighted mean germination time, where germination counts serve as weighting factors. Where, f_i , number of seeds germinated on day i (germination count), and x_i , days elapsed from sowing to observation day i (time interval).

Corrected Germination Velocity Coefficient (GVC_c):

$$GVC_c = \frac{GVC}{G_g} \quad (5)$$

This study used the coefficient proposed by Kotowski (1926), corrected by the total number of germinated seeds (G_g) according to Santana & Ranal (2004), where CVG_c represents the Corrected Germination Speed Coefficient.

Seedling performance-based procedures were also applied for the greenhouse seedling emergence test.

The experiment followed a completely randomized design, and data were subjected to analysis of variance. For data not conforming to normal distribution, we applied the arcsine square root transformation [$\arcsin\sqrt{x/100}$]. Means were compared using Tukey's test at 5% probability level employing R software (R Core Team, 2023).

Results and discussion

The seed water content reached values of 7.6% for Lot 1 and 8.1% for Lot 4, showing a variation of 0.5 percentage points. Small variations in water content provide greater credibility and do not compromise result interpretation, since the variation remained within two percentage points (Ornellas et al., 2020).

The seed lots showed high germination performance, with Lot 1 at 69%, Lot 2 at 90%, Lot 3 at 89%, and Lot 4 at 92%. Only Lot 1 differed from the other treatments, showing lower germination (**Table 1**), and presented normal seedling percentages below the 80% minimum established for commercial melon seed marketing.

In the first count test, as in the germination test, Lot 1 was classified as the lowest vigor lot due to its lower number of normal seedlings, while the other lots showed higher vigor with greater seedling development (**Table 1**).

For electrical conductivity, results showed no differences between treatments, as all lots exhibited similar leachate release patterns with values ranging from 88 to 96 $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$. Torres (2005) reported values of 62.9 to 112.6

$\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$, associated with seeds of high physiological quality of two melon hybrids. Seed classification in this test can be considered high vigor since lower EC values indicate less compromised membrane systems, restricting leachate release into the imbibition solution. Typically, prolonged storage, improper conditions, physical stresses, and other factors are associated with seed deterioration processes that reduce seed vigor. However, the EC test's efficiency for melon seeds has been questioned. Torres et al. (2009) attribute this to the presence of a nucellar-derived semipermeable membrane that likely permits water uptake but restricts electrolyte leakage, potentially making this method unreliable for melon seeds.

Seedling growth can be assessed through both size measurements and seedling mass (or partial mass). Based on root dry matter mass values, Lots 3 and 4 were classified as more vigorous, showing averages of 0.103 g and 0.091 g of dry mass respectively (**Table 1**). Lot 3 exhibited the best primary root development.

The root dry mass and root length measurements showed no significant differences among the four evaluated lots. Hypocotyl measurements classified Lot 1 as least vigorous (48 mm length), while other lots ranged between 56-60 mm (**Table 1**). Seedling length, an important performance-based test, effectively distinguished vigor in alfalfa seed lots (Pan et al., 2025).

Seedling emergence in greenhouse conditions underperformed compared to laboratory germination tests conducted under optimal conditions. While Lots 3 and 4 showed 89-92% germination, their emergence averaged 87% (**Table 1**). Interestingly, Lot 1's poor laboratory performance didn't translate to poor greenhouse development. When environmental conditions differed from laboratory standards (optimal uniform temperature and humidity), lot performance equalized, showing no differences. Lot 1, despite lower physiological quality, could establish adequate field populations when sown in proper quantity, timing, and location - just as vigorous seeds might show poor field emergence (Ebene, 2019).

Germination velocity represents one of the oldest seed vigor evaluation concepts. While different seed lots may achieve similar final germination percentages, their germination rates can vary significantly. This temporal heterogeneity necessitates multiple germination speed indices to properly classify seed lots based on uniformity of germination timing. In our study, four established parameters - Germination Speed Index (GSI), Corrected GSI (GSIc), Corrected GSI version 2 (GSIc2), and Germination Velocity Coefficient (GVC) (**Table 2**) -

consistently identified Lot 1 as the least vigorous and Lot 4 as the most vigorous. This classification pattern showed strong agreement with the final germination percentages observed across the melon seed lots, confirming the complementary value of kinetic parameters in comprehensive vigor assessment.

The emergence speed index under greenhouse conditions showed strong correlations with root length measurements. Seedling length effectively distinguished vigor levels among soybean seed lots, demonstrating its value as a key parameter for evaluating seed physiological potential (Araújo, 2021).

The Corrected Germination Velocity Coefficient by germinated plants (GVCC) revealed no significant differences among the evaluated seed lots (Table 3). Similarly, greenhouse seedling emergence tests showed no statistically significant variation between lots (Table 1).

The germination speed indices proposed by Maguire (1962) are widely employed in seed quality assessments: Pan et al. (2025) applied these to alfalfa seeds; Silva et al. (2025) evaluated genipap seeds under silicon treatment and salt stress; Barbosa et al. (2016)

assessed peanut seed quality; and Ribeiro-Oliveira & Ranal (2016) studied Brazilian Cerrado native species. These studies collectively demonstrate that higher index values correlate with faster germination rates and consequently greater seed vigor, as vigorous seeds exhibit both enhanced stress tolerance and quicker imbibition/reactivation toward germination.

While Maguire's (1962) formula remains more prevalent than Kotowski's (1926) approach, the standard Germination Speed Index (GSI) may fail to distinguish between lots with subtle vigor differences (Nakagawa, 1999). Correction-factor formulas (GSI_c, GSI_{c2}) address this by normalizing for final germination percentages, proving particularly valuable in stress studies (e.g., temperature, salinity, water deficit) where partial germination inhibition occurs but viable seeds still show vigor differences. This aligns with findings in melon seeds under low-temperature and water stress conditions, where germination timing effectively reflected vigor (Kaya, 2024).

The results demonstrate that germination speed indices (GSI, GSI_c, GVC) obtained under controlled conditions showed extremely strong correlations ($r > 0.98$;

Table 1. Water content (WC), germination (GE), first count (FC), electrical conductivity (EC), root dry matter mass (RDM), hypocotyl length (HL), root length (RL), and seedling emergence in greenhouse (SE) of yellow Valenciano-type melon seeds

Lots	WC	GE	FC	EC	RDM	HL	RL	SE
		----- % -----		$\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$	$\text{g}\cdot\text{plant}^{-1}$	mm	mm	%
1	7.6	69 b ¹	64 b ²	96 a	0.089 a	48 b	95 a	70 a ²
2	8.0	90 a	78 a	94 a	0.093 a	56 ab	110 a	79 a
3	7.7	89 a	73 a	88 a	0.103 a	60 a	108 a	87 a
4	8.1	92 a	78 a	92 a	0.091 a	56 ab	93 a	87 a
CV (%)	-	8.3	5.5	6.3	8.8	8.5	12.6	9.2

¹Means followed by the same letter in a column do not differ significantly by Tukey's test ($p < 0.05$). ²Original data shown - statistical analysis performed on transformed data

Table 2. Germination kinetic parameters of yellow Valenciano-type melon (*Cucumis melo* L.) seeds: Germination Speed Index (GSI), Corrected Germination Speed Index (GSI_c), Corrected Germination Speed Index 2 (GSI_{c2}), Germination Velocity Coefficient (GVC), and Corrected Germination Velocity Coefficient (GVC_c).

Lots	GSI	GSI _c	GSI _{c2}	GVC	GVC _c
1	17 b ¹	0.415 b ²	0.334 b	35.9 b ²	0.898 a
2	22 a	0.458 a	0.438 a	42.3 ab	0.884 a
3	21 a	0.457 ab	0.418 a	42.1 ab	0.921 a
4	22 a	0.467 a	0.446 a	43.8 a	0.916 a
CV (%)	6.6	2.25	6.56	5.26	9.75

¹Means followed by the same letter in a column do not differ significantly by Tukey's test ($p < 0.05$). ²Original data shown - statistical analysis performed on transformed data.

Table 3. Emergence kinetic parameters of yellow Valenciano-type melon (*Cucumis melo* L.) seeds: Emergence Speed Index (ESI), Corrected Emergence Speed Index (ESI_c), Corrected Emergence Speed Index 2 (ESI_{c2}), Emergence Velocity Coefficient (EVC), and Corrected Emergence Velocity Coefficient (EVC_c).

Lots	ESI	ESI _c	ESI _{c2}	EVC	EVC _c
1	7 a ^{1,2}	0.187 a ²	0.156 a	17,288 a	0.839 a
2	7 a	0.198 a	0.166 a	19,506 a	0.884 a
3	8 a	0.207 a	0.191 a	19,788 a	0.862 a
4	9 a	0.213 a	0.194 a	20,219 a	0.890 a
CV (%)	17.4	6.3	12.9	7.2	15.1

¹Means followed by the same letter in a column do not differ significantly by Tukey's test ($p < 0.05$). ²Original data shown - statistical analysis performed on transformed data.

p < 0.05) with both germination percentage (GER) and first count (FC) (Figure 1A). These findings confirm their reliability as laboratory vigor assessment tools. The corrected index (GSIc) showed particular sensitivity in discriminating subtle differences between seed lots (r = 0.996 with GER), enabling early physiological quality prediction (3-5 days) prior to conventional test completion. The high GVC-GER correlation (r = 0.994) further establishes germination velocity as a robust parameter for rapid quality control screening.

In contrast, field emergence speed indices (SE

variants) showed no significant correlations with most laboratory parameters (Figure 1B), except for a moderate association with hypocotyl length (HL; r = 0.908, p ≈ 0.09). This suggests environmental factors (e.g., soil moisture, uncontrolled temperature) mediate the relationship between intrinsic vigor and field performance. The weak SE-GSI correlation (r = 0.821; p = 0.18) demonstrates that laboratory germination rates don't ensure uniform field emergence, underscoring the need to either: i) Complement laboratory tests with field evaluations, or, ii) Develop environmentally-adjusted indices incorporating local variables.

Conclusion

Seedling performance-based tests proved effective for evaluating melon seed vigor exclusively under controlled laboratory conditions.

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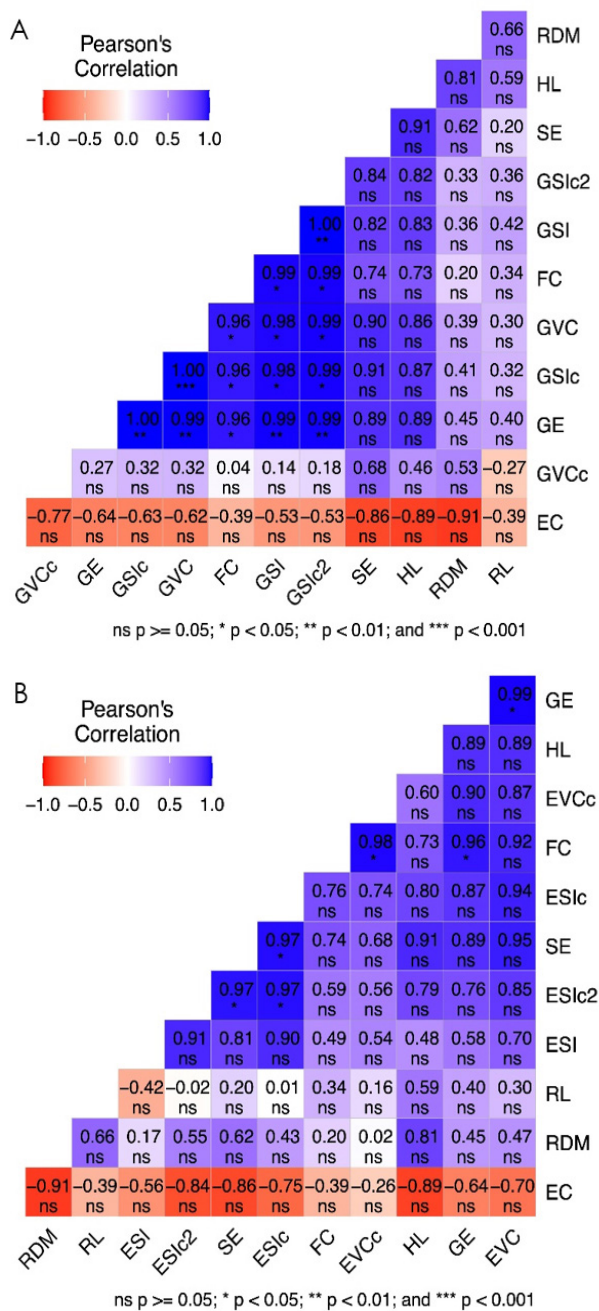


Figure 1. Pearson correlation analysis of physiological quality assessment methods for melon (*Cucumis melo* L.) seeds: (A) Laboratory-based seedling performance metrics, and (B) Greenhouse-based seedling emergence parameters.

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