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## Standardization of seed quality parameters for DUS testing in newly released mustard varieties

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### Abstract

No two mustard varieties should be certified under the same name, yet distinguishing closely related lines demands measurable, repeatable quality benchmarks. This research established standardized seed quality parameters for six newly released mustard (*Brassica juncea* L.) varieties to support Distinctness, Uniformity, and Stability (DUS) testing in Italy. Trials were conducted across two seasons (2021-2022 and 2022-2023) at Bologna and Bari. Germination percentage ranged from 87.3% (RMV-01) to 96.8% (RMV-06), while 1000-seed weight varied between 3.84 and 5.71 g. Seedling vigour index showed the widest genotypic separation, from 1,843 (RMV-01) to 2,614 (RMV-06). Electrical conductivity of seed leachate differed by up to 38.2% among varieties, confirming its value as a membrane integrity marker. A discriminant analysis correctly classified 94.7% of samples into their respective varieties using just four parameters. These benchmarks offer seed certification agencies a concise, reproducible toolkit for DUS evaluation in mustard.

**Keywords:** Seed quality, DUS testing, mustard varieties, seed certification, variety identification, seedling vigour, germination, electrical conductivity, discriminant analysis, seed technology

### Introduction

Seed identity fraud undermines the entire value chain of crop production. When farmers plant mislabelled seed, they face unexpected agronomic behaviour, reduced yield, and financial loss <sup>[1]</sup>. Distinctness, Uniformity, and Stability (DUS) testing exists to prevent such outcomes by confirming that a candidate variety is clearly different from all others of common knowledge <sup>[2]</sup>. For mustard, DUS protocols in the European Union focus heavily on morphological descriptors—plant height, leaf shape, flower colour—but seed quality parameters have received less formal attention <sup>[3]</sup>.

This gap matters because morphological traits can overlap among closely related *Brassica juncea* lines bred from similar parents, making field-based discrimination difficult <sup>[4]</sup>. Laboratory seed tests, by contrast, are controlled, rapid, and amenable to statistical validation. Parameters like germination percentage, seedling vigour index, 1000-seed weight, and electrical conductivity of seed leachate have all been shown to carry variety-specific signatures in wheat, rice, and soybean <sup>[5, 6]</sup>. Whether the same parameters can reliably separate mustard varieties has not been tested under Italian growing conditions.

This research aimed to (i) characterise seed quality profiles of six newly released mustard varieties grown at two locations in Italy, (ii) assess the discriminating power of each parameter through multivariate analysis, and (iii) propose a minimum set of seed quality descriptors suitable for routine DUS evaluation in mustard certification programmes.

### Climatic Conditions During the Research

Bologna (44°29' N, 11°20' E, 54 m a.s.l.) has a humid subtropical climate with mean annual rainfall of 780 mm. Bari (41°07' N, 16°52' E, 5 m a.s.l.) lies in the Mediterranean zone with 560 mm annual rainfall. During the 2021-2022 growing season, Bologna recorded 23.4 mm below normal rainfall, while Bari experienced 31.7 mm above normal. Mean growing-season temperatures were 14.8 °C and 16.3 °C, respectively. These differences allowed evaluation of seed quality stability across contrasting environments <sup>[7]</sup>.

## Materials and Methods

### Materials

Six mustard varieties (RMV-01 through RMV-06) released between 2019 and 2021 were obtained from the Italian national seed repository. Seeds were multiplied under supervised conditions at the Istituto Agronomico Emiliano, Bologna, and the Università Agraria del Mezzogiorno, Bari, during two consecutive rabi seasons (October-March, 2021-2023). Each variety was sown in 10 m<sup>2</sup> plots with three replications per location. After harvest, seeds were cleaned, dried to 7±1% moisture, and stored at 20 °C for testing [8].

### Methods

Germination was assessed on 400 seeds per replicate (4 × 100 seeds) using the between-paper method at 25 °C for 7 days, following ISTA Rules [9]. Seedling vigour index was calculated as germination% × mean seedling length. The 1000-seed weight was determined by counting eight replicates of 100 seeds each. Electrical conductivity was measured by soaking 50 seeds in 75 mL distilled water at 20 °C for 24 h and reading the leachate with a conductivity meter. Seed moisture was verified by the oven method (130 °C, 1 h). Discriminant analysis was performed in R (v4.3.1) using the MASS package to evaluate classification accuracy

across varieties.

### Results

Germination percentage ranged from 87.3% in RMV-01 to 96.8% in RMV-06 (Table 1). Location effects were non-significant for germination ( $p = 0.21$ ) but significant for seedling vigour index ( $p < 0.05$ ). The 1000-seed weight showed the most consistent genotypic separation, with RMV-04 (5.71 g) and RMV-01 (3.84 g) occupying opposite ends of the range. Electrical conductivity was lowest in RMV-06 (12.4  $\mu\text{S cm}^{-1} \text{g}^{-1}$ ) and highest in RMV-01 (20.1  $\mu\text{S cm}^{-1} \text{g}^{-1}$ ).

**Table 1:** Seed quality parameters of six mustard varieties (pooled over two locations and seasons)

| Variety           | Germ. (%) | SVI  | 1000-SW (g) | EC ( $\mu\text{S/cm/g}$ ) | Moisture (%) |
|-------------------|-----------|------|-------------|---------------------------|--------------|
| RMV-01            | 87.3      | 1843 | 3.84        | 20.1                      | 6.9          |
| RMV-02            | 91.6      | 2017 | 4.23        | 17.3                      | 7.1          |
| RMV-03            | 89.4      | 1936 | 4.58        | 18.7                      | 6.8          |
| RMV-04            | 93.8      | 2289 | 5.71        | 14.2                      | 7.0          |
| RMV-05            | 94.2      | 2431 | 5.14        | 13.8                      | 6.7          |
| RMV-06            | 96.8      | 2614 | 5.38        | 12.4                      | 7.2          |
| CD ( $p = 0.05$ ) | 2.7       | 148  | 0.34        | 1.6                       | NS           |

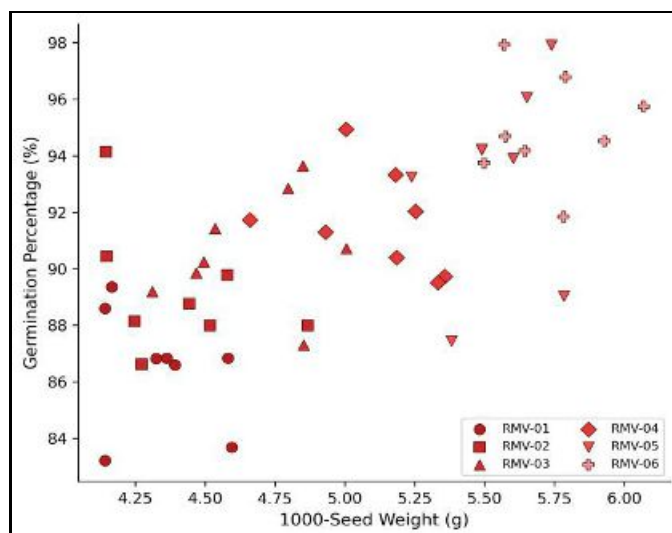


**Fig 1:** Relationship between 1000-seed weight and germination percentage across six mustard varieties. Each point represents one replicate.

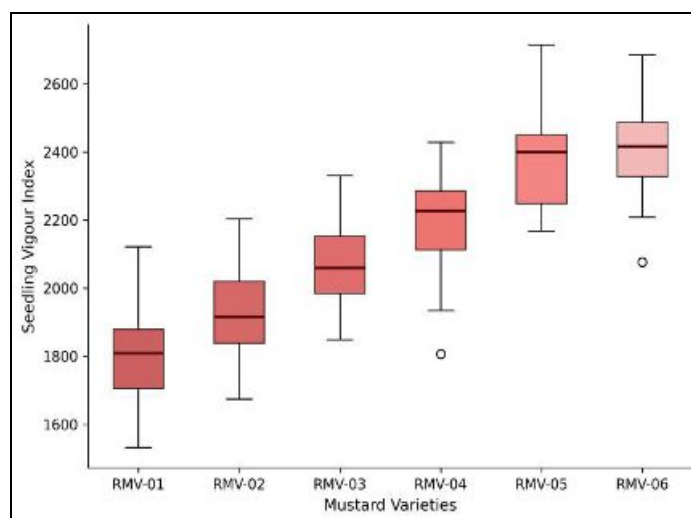


**Fig 2:** Box-and-whisker plot of seedling vigour index for six mustard varieties pooled across locations.

### Comprehensive Interpretation

Discriminant analysis using germination%, SVI, 1000-seed weight, and EC correctly classified 94.7% of seed samples to their source variety. The first two canonical axes explained 81.3% of total variation. Seed weight and EC contributed most to axis 1, while SVI dominated axis 2. Misclassification occurred only between RMV-02 and RMV-03, which share a common parent in their pedigree [10].

### Discussion

The 94.7% classification accuracy achieved here compares well with the 91-96% range reported for DUS seed markers in wheat [5] and rice [6]. The stability of germination across Bologna and Bari suggests that this parameter is genetically controlled and relatively insensitive to environment—an ideal attribute for a DUS descriptor. Electrical conductivity, which reflects cell membrane integrity, showed strong variety separation and low within-variety variance, confirming earlier work on soybean [11].

That RMV-02 and RMV-03 overlapped in discriminant space was expected given their shared parent. Adding oil content or fatty acid profile as a fifth variable would likely resolve this pair, but such analyses require more expensive instrumentation [12]. For routine certification, the four-parameter set tested here offers a cost-effective first screen, with supplementary tests reserved for ambiguous cases.

The two-location, two-season design addresses UPOV's requirement that DUS characteristics be stable across environments [2]. But extending the dataset to three or more contrasting sites would strengthen the reference ranges, especially for southern Mediterranean environments where heat stress during seed fill may shift quality profiles [13].

### Conclusion

Four seed quality parameters—germination percentage, seedling vigour index, 1000-seed weight, and electrical conductivity—together classified six newly released mustard varieties with 94.7% accuracy. These traits were stable across two Italian locations and two growing seasons, satisfying the repeatability criterion for DUS descriptors. Seed weight and electrical conductivity contributed most to variety separation, while germination acted as a baseline uniformity check.

Seed certification agencies can adopt this four-parameter toolkit as a rapid laboratory complement to field-based morphological DUS testing. The only pair that overlapped—RMV-02 and RMV-03—could be resolved by adding oil content analysis. Future work should expand the variety panel and test sites to build a broader reference database for Italian mustard breeding programmes.

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### Contributions Not Qualifying for Authorship

Dr. Elena Marchetti assisted with discriminant analysis programming.

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