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Root protrusion as a vigor test for canola seeds under different levels of water availability

Protrusão radicular como teste de vigor em sementes de canola sob diferentes níveis de disponibilidade hídrica

Aline Ulzefer Henck[1]*; Patrícia Pivetta[2]; Ubirajara Russi Nunes[3]; Iuri Coletto Balensiefer[4]; Cassio Miguel Ferrazza[5]

Highlights

Higher water availability resulted in increased radicle protrusion rates.
Longer assessment times led to higher radicle protrusion rates.
Radicle protrusion test is a feasible method to assess vigor in canola seeds.

Abstract

Canola is used to produce oil for human consumption, biodiesel, and as part of crop rotation systems. This study aimed to determine whether radicle protrusion can be recommended as a vigor test for canola seeds. The experiments were conducted at the Federal University of Santa Maria, using seeds from the canola hybrids Ceres IMI and Nuola 300. Water content and 1000-seed weight were evaluated and vigor and germination tests performed. Primary root protrusion was evaluated in sand at four different water-holding capacities (80%, 60%, 40% and 20%) and four assessment times (24 h, 48 h, 72 h and 96 h). The experimental design was completely randomized, in a 4 x 4 factorial scheme with four replicates, totaling 64 experimental units. Data were analyzed using analysis of variance (ANOVA), polynomial regression, mean comparison tests, and Pearson's correlation at 5% significance, in R Studio software. The radicle protrusion test is a practical and cost-effective alternative for evaluating vigor in canola seeds and should be conducted in a substrate with 60% water-holding capacity in sand, with assessments at 72 hours.

Key words: *Brassica napus* L. Quality control. Primary root.

[1] Doctoral Student in the Postgraduate Program in Agronomy, Universidade Federal de Santa Maria, UFSM, Santa Maria, RS, Brazil. E-mail: alineulzeferhenck@gmail.com

[2] Mester Student in the Postgraduate Program in Agronomy, UFSM, Santa Maria, RS, Brazil. E-mail: pivettapatricia2@gmail.com

[3] Prof. Dr., UFSM, Santa Maria, RS, Brazil. E-mail: urussinunes@gmail.com

[4] Mester Student in the Postgraduate Program in Agronomy, UFSM, Santa Maria, RS, Brazil. E-mail: iuri.balensiefer@acad.ufsm.br

[5] Doctoral Student in the Postgraduate Program in Agricultural Engineering, UFSM, Santa Maria, RS, Brazil. E-mail: cassiom.ferrazza@gmail.com

* Author for correspondence

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Resumo

A canola é empregada na produção de óleo para consumo humano, biodiesel e para compor os sistemas de rotação de cultura. O objetivo desse trabalho foi verificar se a protrusão radicular pode ser indicada como teste de vigor para sementes de canola. Os experimentos foram conduzidos na Universidade Federal de Santa Maria, utilizando sementes dos híbridos de canola Ceres IMI e Nuola 300. Foram avaliados o teor de água, peso de mil sementes, e realizados testes de germinação e vigor. A protrusão da raiz primária foi avaliada sob 4 distintas capacidades de retenção em areia (80%, 60%, 40% e 20%) e quatro tempos de contagem (24 h, 48 h, 72 h e às 96 h). O delineamento utilizado foi o inteiramente casualizado, com 4 repetições, em esquema fatorial 4 x 4, totalizando 64 unidades experimentais. Os dados foram analisados por meio de análise de variância (ANOVA), regressões polinomiais, testes de médias e correlação de Pearson, utilizando o software R Studio, com significância de 5%. O teste de protrusão radicular é uma alternativa prática e econômica para avaliar o vigor de sementes de canola, devendo ser realizada em substrato com 60% de capacidade de retenção em areia e avaliações às 72 horas.

Palavras-chave: *Brassica napus* L. Controle de qualidade. Raiz primária.

Introduction

Canola (*Brassica napus* L.) is an oilseed crop belonging to the family Brassiaceae and originated through rapeseed (colza) breeding (Cardoso et al., 2023). Global canola production is approximately 87.2 million metric tons and the main producers are Canada, China and India (Food and Agriculture Organization of the United Nations [FAOSTAT], 2024). According to the Companhia Nacional de Abastecimento [CONAB] (2024), the country's most recent harvest yielded 146,500 metric tons of grain, with an average yield of 1,591 kg ha^{-1} cultivated across 92,100 ha, concentrated specifically in the southern states of Paraná and Rio Grande do Sul.

This oilseed is an important crop on the global market due to its use in oil production for human consumption and biodiesel. Its high profitability has driven increased cultivation in Brazil (Lima et al., 2017), It has also emerged as a viable winter crop, playing a crucial role in crop rotation systems (Ludwig et al., 2023). However, canola is subject to biotic and abiotic stresses throughout its ontogenetic cycle, water stress being one of the most detrimental to seed vigor and germination (Ávila et al., 2007; Santos et al., 2012).

Using seeds of high physiological quality is essential for achieving high yields (Diaguna et al., 2024). According to Rego et al. (2023), seed physiological quality is typically assessed using laboratory germination tests, which provide reliable data on seed viability. However, obtaining the results takes considerable time and because these tests are conducted under ideal environmental conditions, they may overestimate seed physiological potential. On the other hand, vigor tests are faster, more precise, and less costly.

As reported by I. C. Oliveira et al. (2019), the radicle protrusion test is a vigor assessment method based on the principle that high-vigor seeds emit the primary root more rapidly than their low-vigor counterparts. In viable seeds, primary root emergence is the final phase of germination and occurs after imbibition and a series of biomechanical changes (Dias et al., 2019).

Recent studies have evaluated and confirmed the effectiveness of the radicle protrusion test in the seeds of various crops, including chia (I. C. Oliveira et al., 2019), coffee (Trujillo et al., 2019), tomato (G. R. F. Oliveira et al., 2021), soybean (Rego et al., 2023), and sorghum (Diaguna et al., 2024). However, its validity for other agriculturally important crops, such as canola, has yet to be sufficiently explored, highlighting the need for further research. Therefore, the aim of this study was to determine whether radicle protrusion can be recommended as a vigor test in canola seeds, under varying water retention capacities in sand and at different assessment times.

Material and Methods

The experiments were conducted at the Seed Research Teaching Laboratory (LDPS) of the Department of Crop Science at the Federal University of Santa Maria, in Santa Maria, Rio Grande do Sul state (RS) (Figure 1). Canola seeds (Brassica napus L. var. oleifera) of the Ceres IMI and Nuola 300 hybrids were used.

Initial seed quality for both hybrids was characterized based on water content and 1000-seed weight. Additionally, germination and vigor tests were carried out, including first count (FC), germination speed index (GSI), root (RL) and shoot length (SL), and seedling dry mass (SDM), followed by two experiments to evaluate primary radicle emergence.

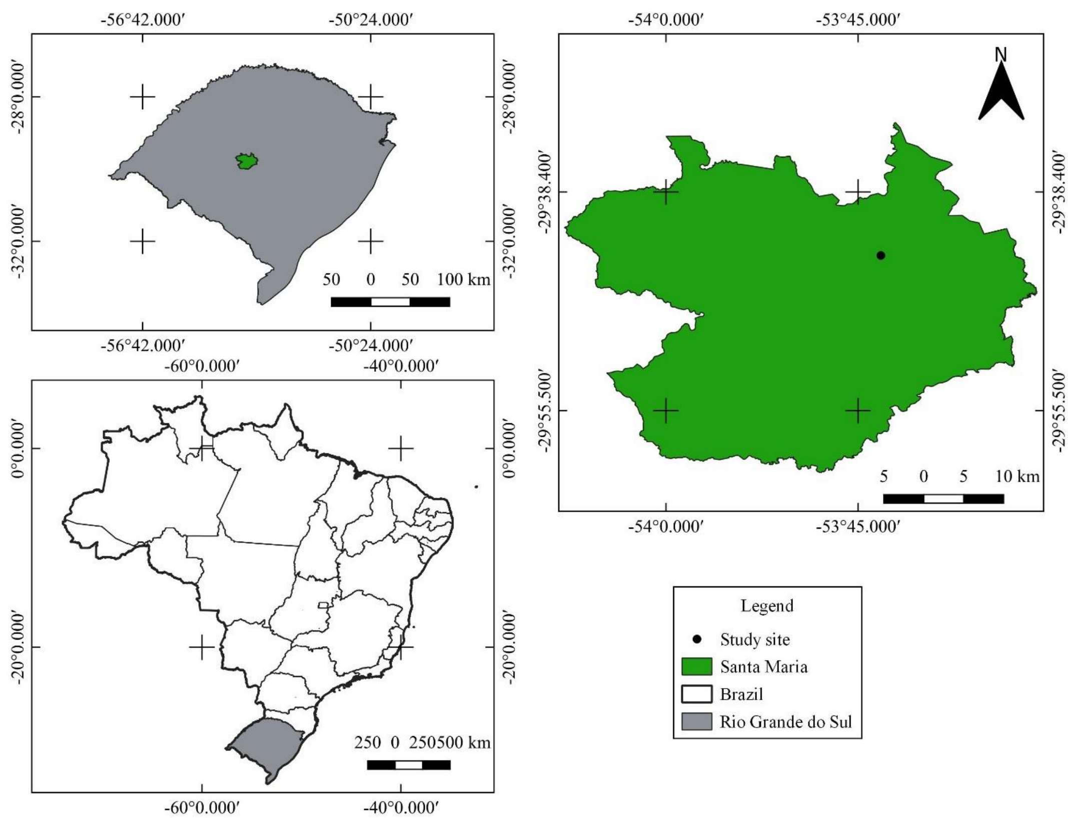

Figure 1. Location of the study site.

Seed water content (WC): determined by the oven-drying method at 105 °C Ministério da Agricultura, Pecuária e Abastecimento [MAPA] (2009), with two replicates of 3 g seed samples. Initially, the containers and their lids were labelled and weighed on a precision scale (tare = *t*). The seed samples were then transferred to the containers, which were weighed again (initial weight = *P*) and placed in an oven at 105 °C ± 3 ºC with the lids resting on top for 24 hours. After drying, the containers were removed, placed in a desiccator and weighed again (final weight = *p*). The results were expressed as the mean percentage (%) of the two samples, ensuring that the difference between replicates did not exceed 5%. Seed water content was calculated using the following equation:

$$WC\ (\%) = 100 \, . \frac{(P - p)}{(P - t)}$$

where *P* = initial weight (container + lid + wet seeds); p = final weight (container + lid + dry seeds); t = tare (weight of the empty container).

1000- seed weight (1000SW): determined by manually counting eight replicates of 100 seeds each, which were then individually weighed on a precision scale. The coefficient of variation and average weight of the samples were calculated, and 1000SW was obtained by multiplying the average weight of the samples by 10, with the results expressed in grams (g) (BRASIL, 2009).

Germination (G): first, all the materials were sterilized with 70% ethanol. Germination testing was conducted with four replicates of 50 seeds, placed in plastic germination boxes (Gerbox®) on three sheets of Germitest® paper towel, moistened with distilled water at 2.5 times the dry weight of the paper. The boxes were sealed with lids and rubber bands, placed in a plastic bag and transferred to a B.O.D. (Biochemical Oxygen Demand) chamber under a 24-hour photoperiod and air temperature of 20 ± 1 ºC. Seedling counts were performed on the 5th and 7th days after test setup. Seeds were considered germinated when the radicle was at least 2 mm in length. Results were expressed as the average percentage of normal seedlings (BRASIL, 2009).

First germination count (vigor) (FC): used as an indicator of seed vigor, FC was performed concurrently with germination testing using the same methodology. The percentage of normal seedlings was determined on the 5th day after test setup (BRASIL, 2009).

Germination speed index (GSI): conducted alongside the germination test, by performing daily counts of germinated seeds until germination stabilized. The GSI was calculated using the equation proposed by Maguire (1962), as follows:

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \cdots + \frac{Gn}{Nn}$$

where G1, G2, … Gn are the number of seeds germinated at the first, second, and final count, and N1, N2, … Nn the number of days from sowing to the first, second, and final count.

Root (RL) and shoot length (SL): determined on the 5th day (first count), when 10 normal seedlings were randomly selected from each of the four replicates. SL was measured from the insertion point at the base of the primary root to the tip of the shoot, and root length from the tip of the primary root to its insertion at the shoot base, using a graduated ruler. Mean results were expressed in centimeters per seedling (cm seedling^{-1}), as described by Krzyzanowski et al. (2020).

Seedling dry mass (SDM): following length measurement, 10 seedlings from each replicate were placed in Kraft paper bags, dried in an oven at 70°C for 48 hours, and then weighed on a precision scale. The dry mass from each replicate was divided by the number of seedlings measured and the results expressed in milligrams per seedlings (mg seedling^{-1}) (Krzyzanowski et al., 2020).

Radicle protrusion (RP): the radicle protrusion test was conducted after initial characterization. RP was evaluated by determining the percentage of seeds with primary roots measuring 2 mm or longer, under four different water-holding capacities (WHCs) in sand: 80%, 60%, 40% and 20%, and at four assessment times: 24 h, 48 h, 72 h and 96 h after test setup. To that end, seeds were sown in plastic trays containing washed, sterilized, and sieved sand (1 kg tray^{-1}). Fifty seeds were placed in 1 cm-deep furrows with 5 cm spacing between rows. The plastic trays were placed in a B.O.D chamber at 20 °C with a constant photoperiod. The experimental design was completely randomized, with four replicates, in 4 x 4 factorial scheme (WHC x assessment time), totaling 64 experimental units.

The data were subjected to analysis of variance (ANOVA) using the F-test, followed by additional analysis with the Scott-Knott test and polynomial regression fitting to determine the degree and best-fitting equations.

A Student's t-test and Pearson's linear correlation analysis were also performed between RP and FC. All analyses were performed using R Studio software, considering a 5% probability level (R Core Team [R], 2023).

Results and Discussion

The Ceres IMI hybrid exhibited a higher water content (8.4%) and 1000SW (4.1 g) than those recorded for Nuola 300 (7.4% and 3.2 g, respectively). These seed moisture values are considered valid, since Marcos (2015) reports a 3% maximum acceptable difference in water content between seed lots, an important factor in ensuring the validity and reliability of subsequent tests. In regard to initial seed quality, a significant difference was only observed for primary root length, which was 2.31 cm longer per seedling in Ceres IMI than in Nuola 300 (1.92 cm per seedling (Figure 2E).

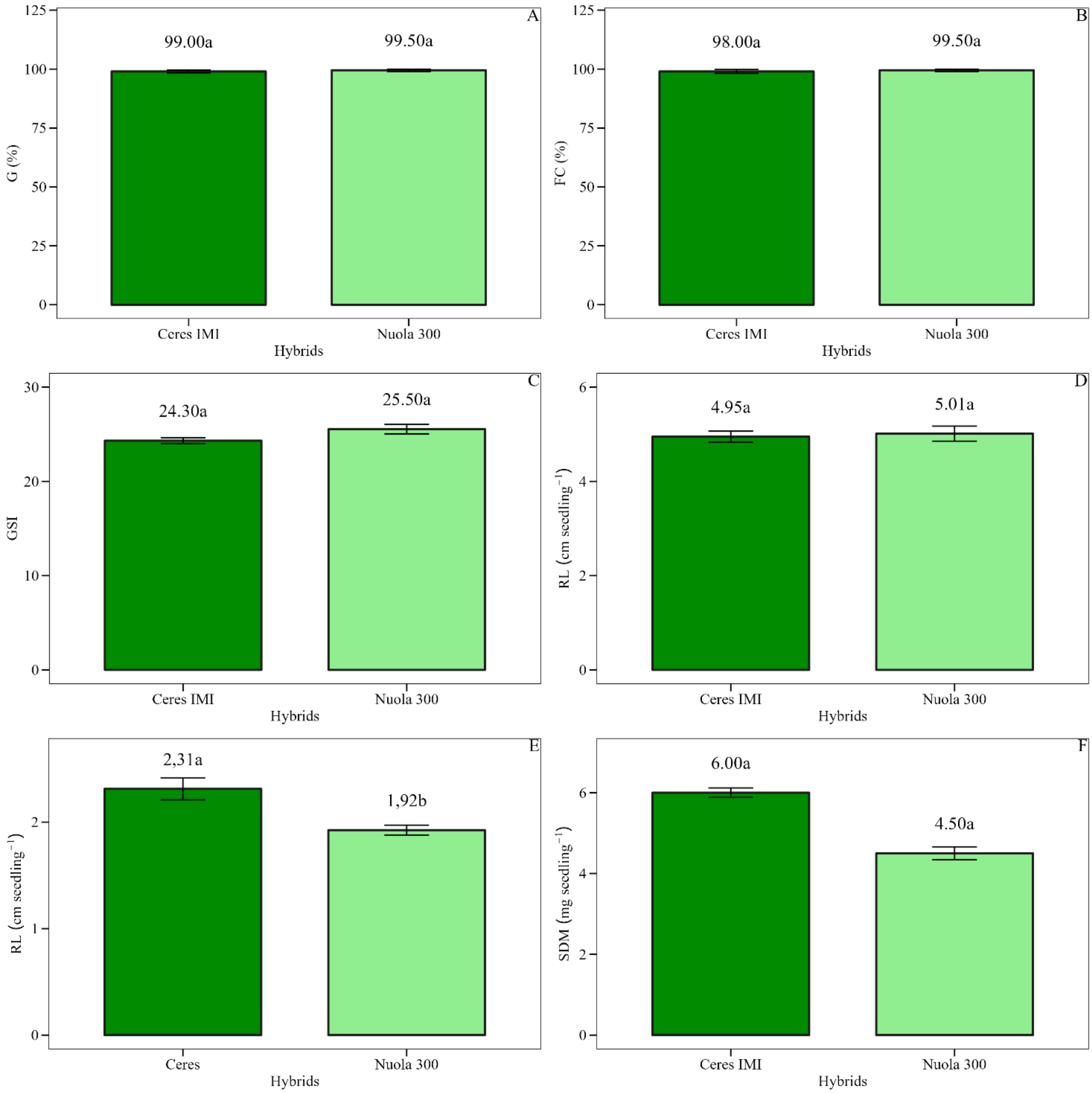

Figure 2. Scott-Knott mean grouping test for the initial quality characterization of two canola seed hybrids based on germination (A), first count (B), germination speed index (C), root length (D), shoot length (E), and seedling dry mass (F).

Both hybrids obtained germination percentages (Figure 2A) and FC results (Figure 2B) within the standards required for commercial canola seed. There were no significant differences between hybrids for GSI (Figure 2C), RL (Figure 2D), or SDM (Figure 2F). Overall, higher means were obtained for Nuola 300, except SDM, which was higher in Ceres IMI.

ANOVA showed a significant interaction ($p < 0.05$) between WHC and assessment times for RP, as well as significant main effects for both factors. Regression analysis enabled the estimation of polynomial equations with quadratic tendencies, except for the curves corresponding to the 20% WHC and 24-hour assessment time, which exhibited no significance.

Distinct RP patterns were observed under varying WHCs for Nuola 300 (Figure 3A) and Ceres IMI (Figure 3B), with higher RP percentages recorded for both regression curves across assessment times. The best results, with RP rates above 80%, were obtained at WHC levels between 60% and 80% for both hybrids and at all assessment times except 24 hours, when RP rates were below 40%.

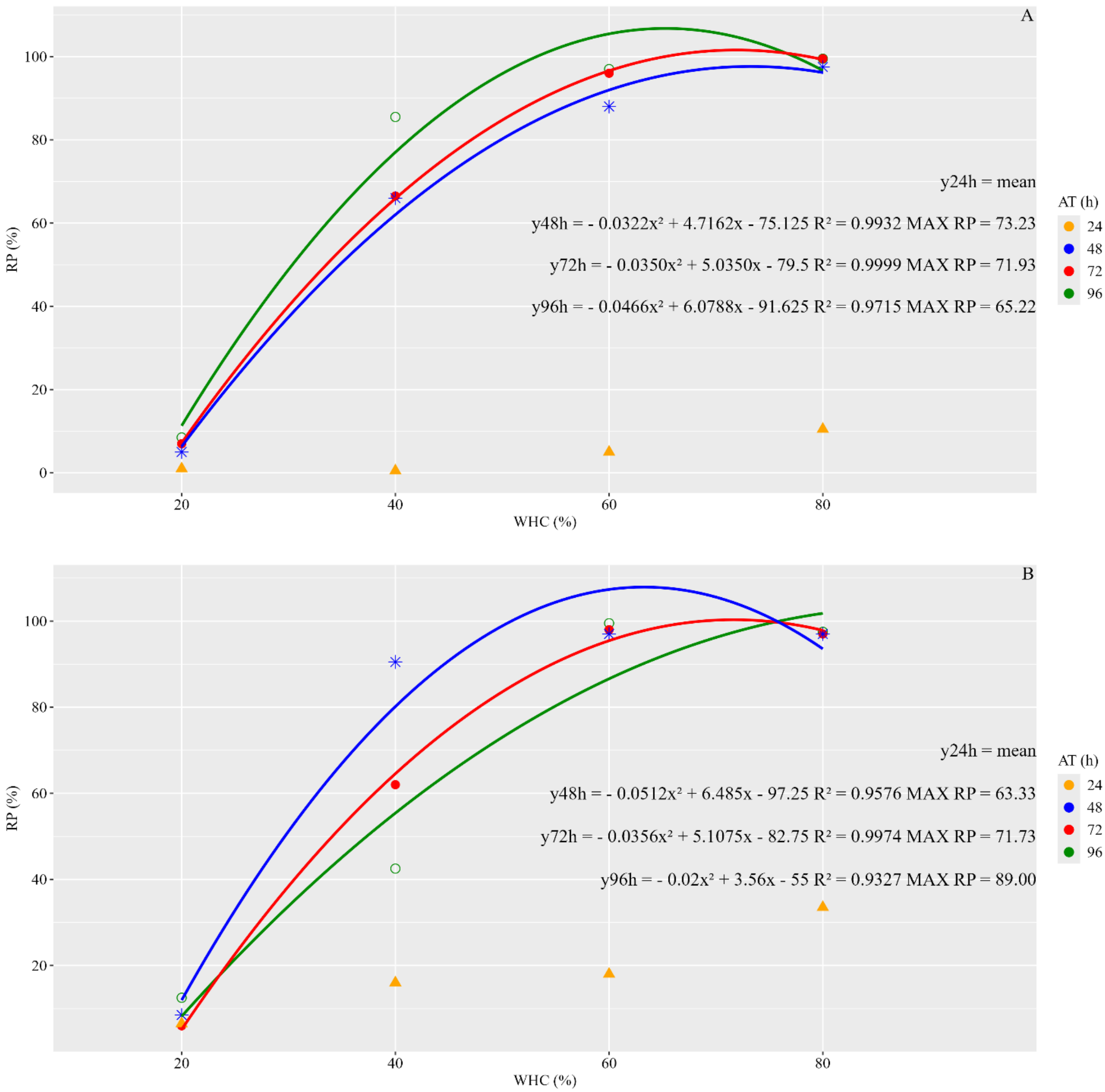

Figure 3. Polynomial regression of radicle protrusion (RP) for the canola hybrids Nuola 300 (A) and Ceres IMI (B) under different water-holding capacities in sand.

Analysis of RP across the different assessment times (ATs) for Nuola 300 (Figure 4A) and Ceres IMI (Figure 4B) indicated that longer evaluation periods led to higher RP rates for both regression curves across WHCs. The best results were recorded at ATs close to 72 hours for both hybrids and across the various WHC levels, except for the 20% WHC curve. At 96 hours, a decreasing trend was observed.

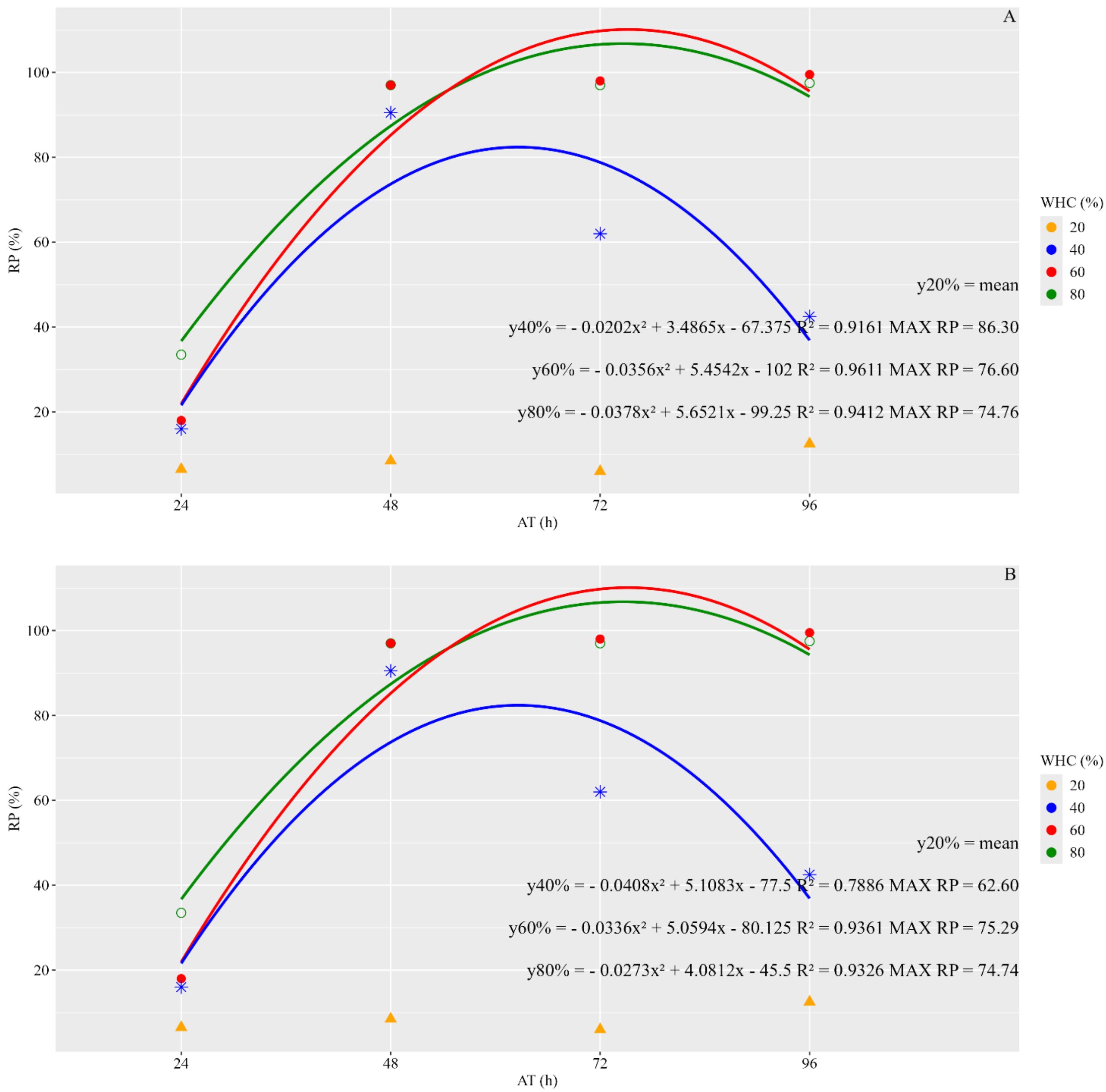

Figure 4. Polynomial regression of radicle protrusion (RP) for the canola hybrids Nuola 300 (A) and Ceres IMI (B) across different assessment times (AT).

Water availability is one of the main factors limiting seed germination and field establishment (Leal et al., 2020; Nunes et al., 2020; Sartori et al., 2023). Under water deficit conditions, reduced water diffusion through the seed coat prolongs the lag phase by lowering enzymatic activity, which in turn limits meristematic development and radicle emergence (Hadas, 1976).

Water deficit can affect seed germination and early seedling development, as recently reported in major crops such as cowpea (Paiva et al., 2018), corn (Sousa et al., 2023), wheat (Sartori et al., 2023) and soybean (Cardoso et al., 2023). In canola, Ávila et al. (2007) observed a significant reduction in germination and seedling performance under water stress. Additionally, Santos et al. (2012) concluded that canola seeds are sensitive to water potentials lower than -0.2 MPa, which compromise seed vigor and germination.

A comparison between the means of each treatment and the reference treatment, considered here to be the first count, indicated that for both Nuola 300 (Figure 5A) and Ceres IMI (Figure 5B), treatments involving 20% WHC regardless of assessment time, and those evaluated at 24 hours regardless of WHC, differed significantly ($p < 0.05$) from the standard. In the case of Nuola 300, it is also important to note that treatments with 40% WHC at 72 h and 60% at 48 h also differed significantly from the reference. Therefore, these treatments should not be used to assess vigor in canola seeds. On the other hand, in all other situations, treatment means were considered statistically equivalent to the first count.

Additionally, RP at 60% WHC showed a positive and significant linear correlation ($p < 0.05$) at 72 h and 96 h for Nuola 300 (Figure 6A) when compared to the values obtained from the first count vigor test, a method recognized by the Brazilian Rules for Seed Testing (RAS) (BRASIL, 2009) and conducted on the 5th day after test setup. For Ceres IMI, this correlation was only observed at 60% WHC and at 72 h (Figure 6B).

The RP test is used to assess seed vigor and potential field performance in various crops. According to Diaguna et al. (2024), the ideal test conditions for sorghum are 28 °C at 36 hours, while for soybean Rego et al. (2023) recommends 30 °C after the same time period, and in chia, I. C. Oliveira et al. (2019) described effective testing at 25 °C and 30 hours.

The RP test is considered a fast and promising method for seed vigor assessment (Silva et al., 2024).

It is based on the premise that the primary root emerges more rapidly in high-vigor seeds than in their low-vigor counterparts (I. C. Oliveira et al., 2019). The primary root emerges in the final phase of germination, after imbibition and several biochemical changes (Dias et al., 2019). Although the RAS does not yet recognize the RP test as a valid method, it proved feasible and efficient for evaluating canola seed vigor in this study, offering advantages such as easy execution and faster results.

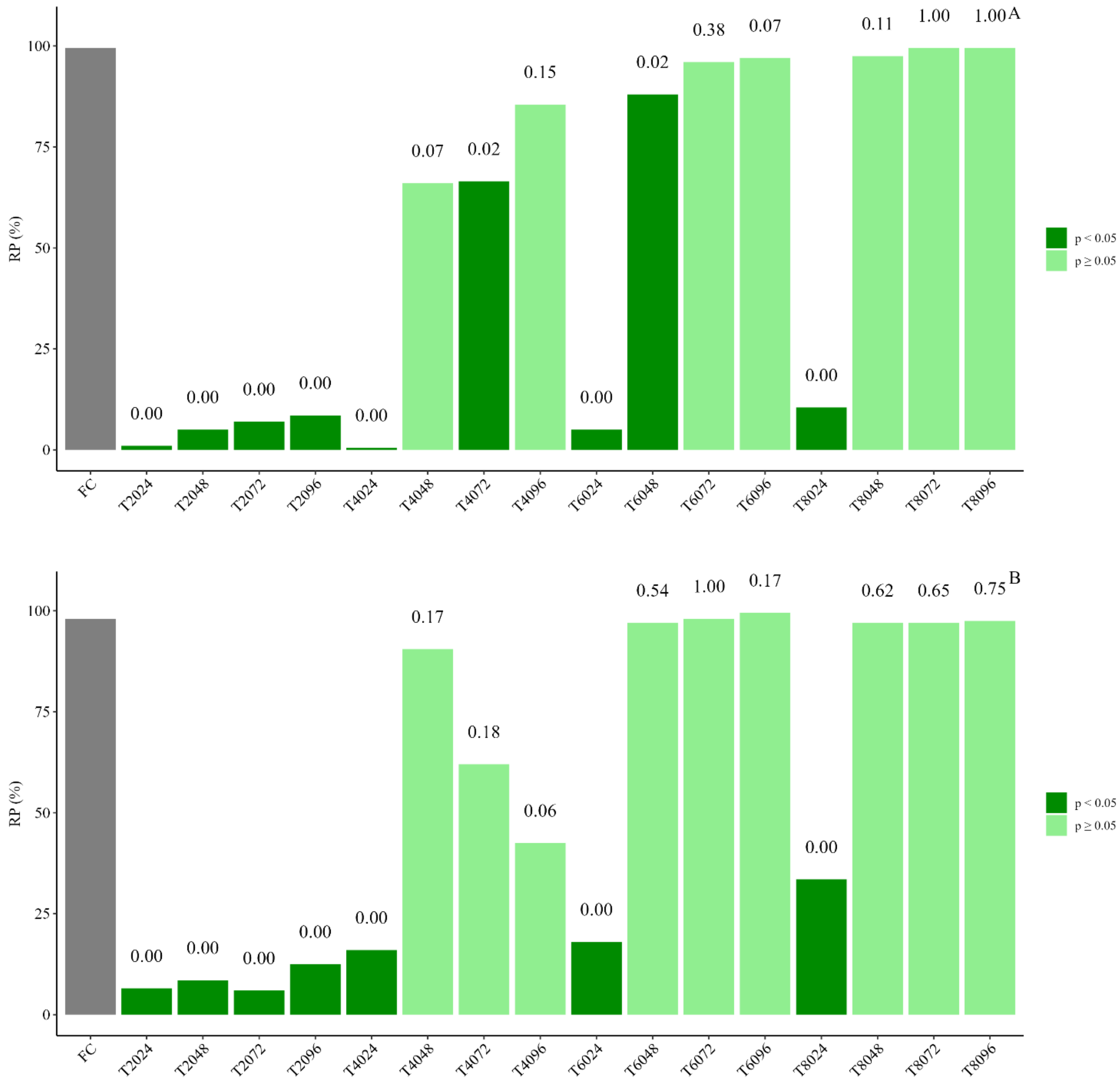

Figure 5. Student's t-test (p-value) comparing the results of the first count (FC) with each treatment for the canola hybrids Nuola 300 (A) and Ceres IMI (B).

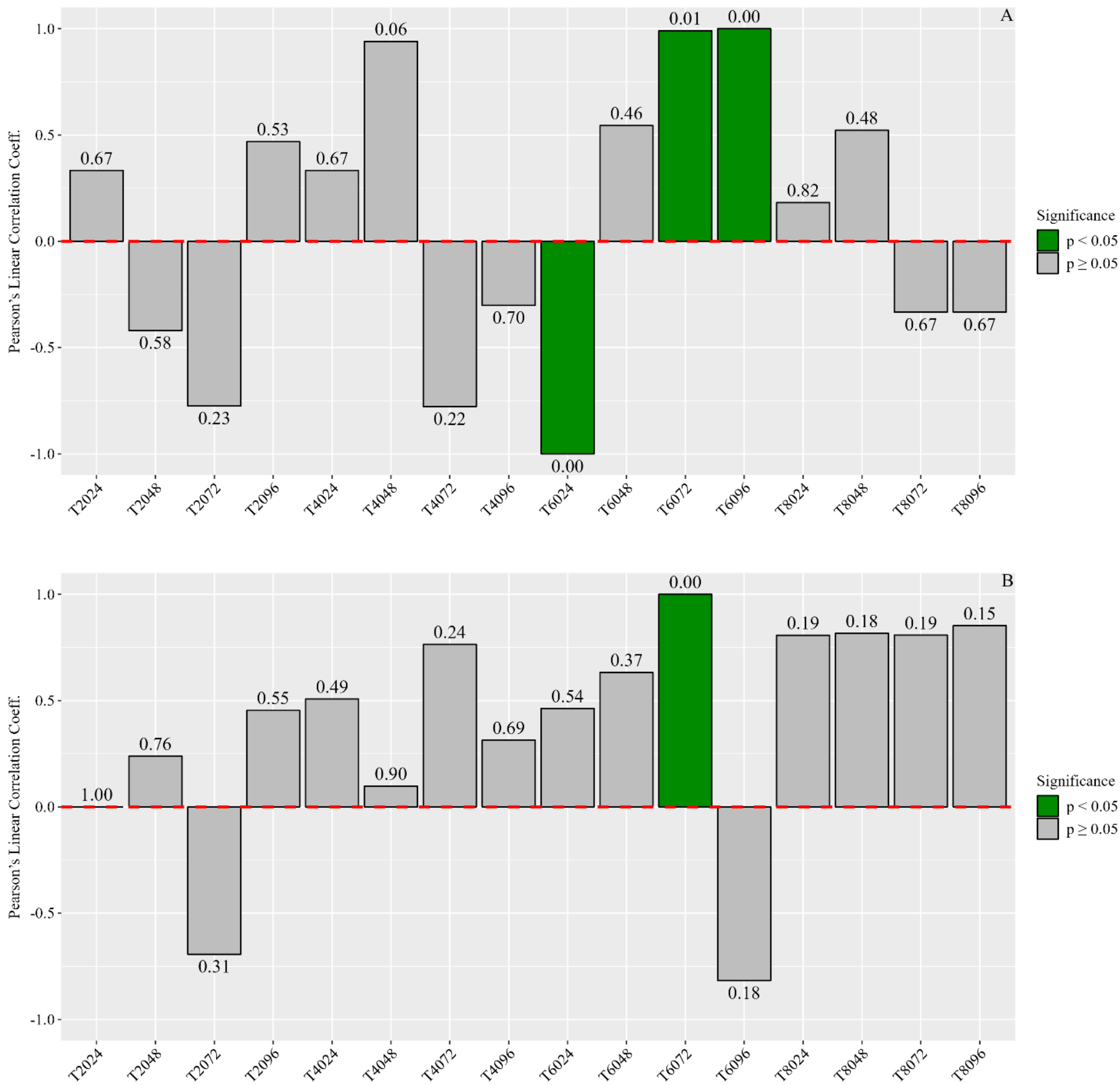

Figure 6. Pearson's linear correlation coefficient and significance (p-value) comparing the results of the first count (FC) with each treatment for the canola hybrids Nuola 300 (A) and Ceres IMI (B).

Conclusions

The radicle protrusion test can be recommended as a vigor test for canola hybrid seeds using a substrate with 60% water-holding capacity and evaluated at 72 hours, offering a practical, rapid, and cost-effective alternative to conventional techniques.

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