

Article

Multivariate Phenotyping of Early Plasticity in *Raphanus sativus* L.: Phenotypic Contrasts in the Morphophysiological Response to In Vitro Fertilization

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Abstract

Seed germination and early root growth are decisive for crop establishment, yet responses to ionic environments can be strongly genotype-dependent. This study evaluated the effect of supplementing an agar-based in vitro system with a commercial NPK fertilizer on the germination dynamics and early seedling traits of *Raphanus sativus* L. Seeds were tested in two solid media: A (1.3% agar, no fertilizer) and AF (1.3% agar supplemented with 0.45 g of granular NPK fertilizer (15–30–15) per 200 mL medium), using a completely randomized 3 × 2 factorial design. Germination percentage and synchrony are key constituents of seedlot evaluation because they jointly capture both viability and the temporal coordination of emergence. However, final germination percentage alone does not reflect the timing and uniformity of germination, which can be critical for predicting establishment and subsequent performance. Therefore, indices such as mean germination time (MGT), coefficient of velocity of germination (CVG), and interval germination rates are frequently employed to describe germination dynamics. In addition to germination dynamics, early seedling morphometry (e.g., root and hypocotyl traits) can provide complementary information on early vigor and stress sensitivity under contrasting media or environmental conditions. Root elongation was significantly reduced by fertilization in ASD and GE, whereas AS exhibited consistently shorter roots with no significant response. PCA summarized 86.3% of the total variance in the first two components, separating treatments along a vigour/architecture axis and a germination capacity axis (%G), and hierarchical clustering identified five response groups. Overall, a low-cost agar + fertilizer system effectively discriminated genotype-specific sensitivity to an ionic environment during early establishment, highlighting the need to consider variety-dependent thresholds when using commercial fertilizers for in vitro screening.

Keywords: in vitro; plant growth; PCA; *Raphanus sativus*; seed germination



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1. Introduction

Seed germination and early seedling establishment represent a critical phase in the plant life cycle because they concentrate largely irreversible physiological decisions—metabolic reactivation, seed-cover weakening, radicle protrusion, and initial tissue differentiation—that ultimately shape seedling vigour and the probability of successful establishment. These processes are governed by the interplay between the internal status of the seed (developmental maturity, reserve mobilization capacity, membrane integrity, and dormancy level) and external drivers such as temperature, water availability, oxygen supply, and the ionic composition of the surrounding medium [1–4].

Among environmental drivers, the physicochemical properties of the germination medium—particularly its osmotic potential and ionic strength—strongly regulate water uptake, metabolic activation, and the rate and uniformity of germination [5]. Therefore, seed performance is typically evaluated not only by final germination but also by the speed and synchrony of emergence, which are closely related to subsequent establishment under practical conditions [6]. In addition, variation among commercial lots and varieties can be amplified by differences in seed ageing and survival, affecting both viability and early performance even before visible emergence occurs [7].

Although final germination percentage is widely used as a quality indicator, it is often insufficient to describe real seed lot performance: different treatments may reach similar final values but exhibit distinct temporal dynamics, translating into differences in synchrony, uniformity, and early vigour. For this reason, methodological frameworks emphasize quantifying the germination process as a time-dependent phenomenon rather than a single endpoint [8]. Likewise, critical evaluations of single-value germination indices show that the shape and dispersion of the cumulative curve contain biologically meaningful information that should be captured through complementary metrics [9]. This is consistent with the broader concept of seed vigour as the capacity to establish rapidly and uniformly, extending beyond mere viability [10].

Early growth—particularly the development of the primary root—constitutes another key component of vigour because it integrates reserve utilization efficiency, cell expansion, and the ability to explore the medium soon after emergence. Foundational plant physiology frameworks highlight that early root elongation is a sensitive readout of growth regulation and resource acquisition potential [11]. Because ionic and osmotic constraints can directly impair cell expansion and water relations, salinity tolerance mechanisms provide an explanatory framework for understanding how ionic stress conditions can penalize early establishment [12]. In parallel, mineral nutrition theory underscores that ions are essential to sustain metabolism and growth, yet imbalanced ionic environments can generate osmotic stress or ion-specific toxicity, compromising germination and seedling development [13,14].

In vitro systems provide a controlled platform to study these processes by minimizing environmental noise and enabling standardized comparisons across genotypes and treatments. Defined culture media have been central to plant tissue culture and early development research, with the Murashige–Skoog formulation serving as a widely adopted reference for controlled growth [15]. Comprehensive tissue culture manuals further emphasize the importance of medium composition and physical support matrices for reproducible development [16]. At the same time, low-cost approaches—including alternative gelling agents and simplified support matrices—have been explored to facilitate large-scale or resource-limited experimentation [17]. However, the interpretability of in vitro assays depends strongly on asepsis: contamination can rapidly alter local pH, oxygen availability, and nutrient dynamics, biasing treatment effects [18], and microbial hazards remain a recurrent challenge in plant tissue and cell culture systems [19].

Accordingly, robust surface-sterilization and establishment protocols are essential to ensure that observed differences reflect treatment and genotype rather than uncontrolled microbial growth. Recent work has demonstrated that optimization of in vitro sterilization and germination conditions benefits from systematic modeling and predictive approaches [20]. From a design standpoint, factorial experiments are especially valuable because they allow separation of main effects (e.g., genotype and fertilization) and their interaction under randomized conditions [21]. When multiple treatments are compared, classical multiple-comparison procedures remain standard for mean separation after ANOVA [22], while rank-based nonparametric approaches provide alternatives when normality assumptions are not met [23,24]. Moreover, controlling error rates under multiple testing is critical to preserve inferential validity, particularly when several indices and response variables are analyzed simultaneously [25].

Beyond univariate inference, early establishment is increasingly interpreted as a multivariate phenotype in which germination dynamics and growth traits covary. Multivariate exploratory tools such as principal component analysis (PCA) and hierarchical clustering can complement univariate tests by summarizing correlated germination and morphometric traits and highlighting treatment groupings [26,27]. Accordingly, we used PCA and clustering to visualize the joint response of radish varieties to contrasting ionic environments [28,29].

Radish (*Raphanus sativus* L.) is frequently used as a rapid and informative model for germination and early growth studies due to its short emergence time and sensitivity to metabolic and environmental modulation, making it well suited for detecting genotype-dependent responses under controlled conditions [30]. To ensure comparability and reproducibility, seed testing standards recommend rigorous sampling procedures and controlled handling, which are particularly relevant when working with commercial seed lots [31]. In addition, reproducible statistical workflows benefit from validated software ecosystems: multivariate analyses are commonly implemented with dedicated R packages for PCA and related methods [32], complemented by visualization tools for extracting and communicating multivariate results [33], all within the R statistical computing environment [34].

Mechanistic and semi-mechanistic modeling further strengthens interpretation of germination dynamics by explicitly linking rate processes to water relations and temperature constraints. Fertilizer addition increases ionic strength and can reduce the effective water potential of the germination medium, creating osmotic and ionic constraints that delay imbibition and radicle protrusion. Therefore, we expected fertilizer-supplemented media to modify germination speed and synchrony, with responses differing among varieties [35–42].

Complementary to modeling, classical indices remain valuable for practical discrimination among treatments and seed lots when applied with appropriate caution. Indices combining speed and completeness, such as germination value, have long been used to integrate performance dimensions into single interpretable measures [43,44]. Moreover, frequent radicle-emergence scoring has been shown to improve the predictive value of time-based metrics for seed vigour, reinforcing the importance of high-resolution temporal data in early establishment studies [45].

In this work, we used three widely cultivated commercial radish (*Raphanus sativus* L.) varieties (Cherry Belle, Sparkler, and Daikon) as contrasting genotypes to test whether fertilizer addition to the germination medium (agar-only vs. agar + NPK) alters germination dynamics and early seedling traits through changes in ionic strength, and whether these responses are variety-dependent. Beyond describing experimental effects, the practical goal is to inform seedlot screening and nursery/propagation management by identifying varieties (or lots) that are more sensitive to high-ionic-strength germination conditions.

This approach provides a rapid, controlled framework to compare early-stage sensitivity to fertilizer-induced ionic environments, which can be used as a practical screening step before greenhouse or field establishment.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

A completely randomized 3×2 factorial design was used, with Variety (Cherry Belle, Sparkler, and Daikon) and Medium (A, agar-only; AF, agar + fertilizer) as fixed factors (Figure 1). For germination and root-length traits, each Petri dish containing nine surface-sterilized seeds was considered one experimental unit; three independent dishes were prepared per treatment combination ($n = 3$), yielding 18 dishes in total. For seedling morphometry, all germinated seedlings per dish (up to 9 seedlings) were measured at 72 h, and their measurements were averaged per dish so that the dish remained the experimental unit for statistical analyses.



Figure 1. Seeds of Agrosad (AS), Agrícola Santo Domingo (ASD), and Garden Elite (GE).

2.2. Experimental Design

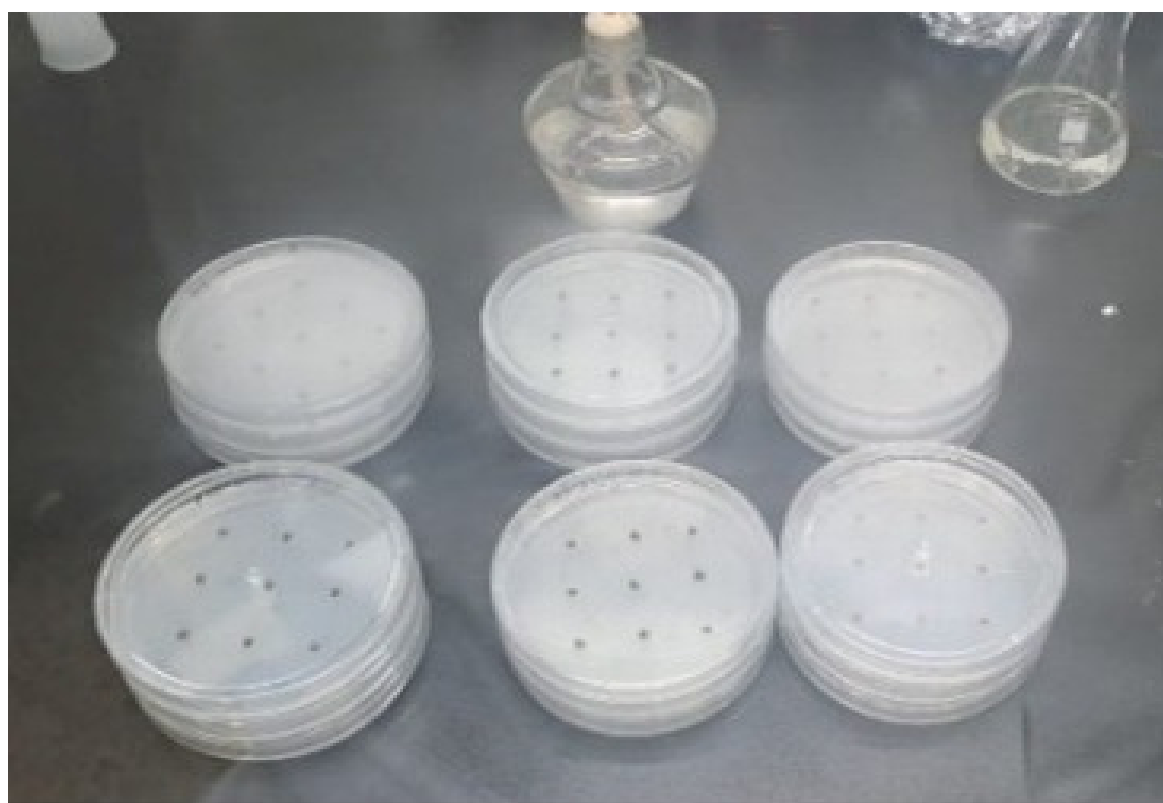
The experiment was conducted under a completely randomized design (CRD) with a 3×2 factorial arrangement, allowing estimation of the main effects of Variety and Fertilization and their interaction through random allocation of experimental units [21]. The design structure and the definition of experimental units by response variable are summarized in Tables 1 and 2, and the sowing layout is illustrated in Figure 2.

Table 1. Structure of the 3×2 factorial design (CRD).

Element	Description
Design type	Completely randomized design (CRD)
Arrangement	3×2 factorial
Factor 1	Variety (3 levels)
Variety levels	AS, ASD, GE
Factor 2	Fertilization (2 levels)
Fertilization levels	A (no fertilizer), AF (supplemented)
Number of treatments	$3 \times 2 = 6$ combinations

Table 2. Experimental units, replication, and sample sizes by response variable.

Response Group/Variable	Unit of Analysis	Unit Size	Replication/Sampling	Per Treatment	Total
Germination (counts and plate-level indices)	Petri dish	9 seeds	3 replicate plates per treatment	3 plates	$6 \times 3 = 18$ plates
Root morphometry (72 h)	Petri dish	All germinated seedlings per plate (up to 9 seedlings)	3 replicate plates per treatment; plate-level mean root length used for statistical analysis	3 plates	$6 \times 3 = 18$ plates

**Figure 2.** Seed sowing arrangement in a 3×3 grid.

2.3. Treatments

Two solid media were prepared to generate contrasting ionic environments: (i) A (agar-only), consisting of 1.3% (*w/v*) agar dissolved in distilled water; and (ii) AF (agar + fertilizer), consisting of 1.3% (*w/v*) agar supplemented with 0.45 g of granular NPK fertilizer (15–30–15) per 200 mL medium. Prior to sowing, seeds were surface-disinfected by immersion in 2% sodium hypochlorite for 10 min, rinsed three times with sterile distilled water, and air-dried under sterile conditions. Plates were sealed with Parafilm and incubated at 25 ± 2 °C in darkness [17,20].

2.4. Assessments and Measured Variables

Interval germination was recorded per replicate at 24, 48, and 72 h as the number of newly germinated seeds in each interval: $G_{24} = n_{0-24}$, $G_{48} = n_{24-48}$, and $G_{72} = n_{48-72}$ (where n denotes the count of newly germinated seeds in the specified interval). For clarity, G_{24} – G_{72} represent interval counts (not cumulative germination percentage); cumulative germination (%) was reported separately as the proportion of germinated seeds relative to the total number of seeds per plate ($N = 9$) (Figure 3).

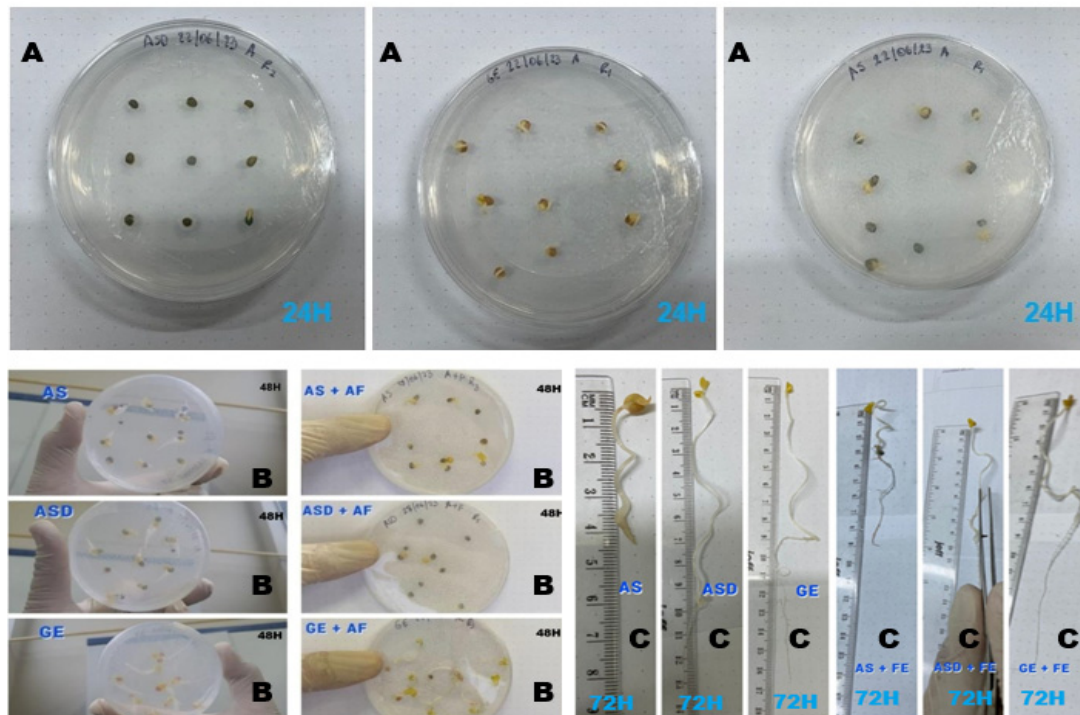


Figure 3. Germination was recorded at 24 (A), 48 (B), and 72 (C) h of incubation. After 72 h, representative seedlings of each variety were photographed to illustrate differences in early root elongation under the evaluated conditions.

2.4.1. Primary Variables

Interval germination (G_{24} , G_{48} , G_{72}) was defined as the number of seeds germinated within each evaluation period: G_{24} (0–24 h), G_{48} (24–48 h), and G_{72} (48–72 h). This interval-based approach characterizes emergence dynamics and discriminates treatments that may reach similar final germination but at different rates.

Root length (72 h) was defined as the length (cm) of the primary root measured at 72 h. For each plate, all germinated seedlings were measured and the plate-level mean root length was used as the experimental unit for statistical analysis. This approach yielded three replicate observations per treatment (18 plates in total) while accommodating treatment-dependent differences in germination.

2.4.2. Calculated Germination-Performance Indices

From interval germination counts, standard germination-performance indices were calculated as follows ($N = 9$ seeds per plate):

Final germination percentage (%G):

$$\% G = \frac{\sum n_i}{N} \times 100$$

where $\sum n_i$ is the total number of germinated seeds accumulated up to 72 h ($G_{24} + G_{48} + G_{72}$).

Mean germination time (MGT): computed following Ellis and Roberts [7]:

$$MGT = \frac{\sum(n_i \cdot t_i)}{\sum n_i}$$

Lower values indicate faster average germination.

Germination speed index (IVG): computed following Maguire [6]. To match the evaluation frequency, time was expressed in days equivalent to 24, 48, and 72 h ($t = 1, 2, 3$):

$$IVG = \frac{G_{24}}{1} + \frac{G_{48}}{2} + \frac{G_{72}}{3}$$

Higher values indicate greater vigour due to earlier and more concentrated emergence.

Coefficient of velocity of germination (CVG): computed following Kotowski [5]:

$$CVG = \frac{\sum(n_i)}{\sum(n_i \cdot t_i)} \times 100$$

where n_i is expressed in hours. Higher values indicate faster germination.

2.5. Statistical Analysis

To ensure traceability between each response variable and the inferential method applied, the statistical approach is summarized in Table 3. Statistical significance was set at $\alpha = 0.05$ for all tests, and multiple-comparison or p -value adjustment procedures were applied when appropriate to control multiplicity [21,25].

Table 3. Summary of statistical analyses by variable.

Variable Group	Variable(s)	Unit of Analysis	Model/Test	Multiple Comparisons/Adjustment
Germination (plate level)	%G, IVG, CVG	Petri dish ($N = 9$ seeds); 3 replicates per treatment	3×2 factorial ANOVA (Variety $\times$ Fertilization)	Tukey HSD (if ANOVA $p < 0.05$) [22]
Germination time	MGT	Plate	Kruskal–Wallis (nonparametric) [23]	Pairwise Wilcoxon with Benjamini–Hochberg adjustment [24,25]
Root morphometry	Root length (72 h)	Plate-level mean root length ($N = 18$ plates)	Independent 3×2 factorial ANOVA (observations not paired by plate) [21]	Tukey HSD (if ANOVA $p < 0.05$) [22]
Multivariate	Standardized variable set	Treatment/observation	PCA on standardized matrix [26–28]	HCPC using Ward + Euclidean distance [28,29]

Specifically, plate-level germination variables (%G, IVG, CVG) were analyzed using a 3×2 factorial ANOVA with three replicates per treatment under a completely randomized design [21]. When significant effects were detected ($p < 0.05$), Tukey’s HSD test was used for mean separation [22]. Because germination and seedling traits are partially correlated, we first applied a two-way MANOVA (Variety, Medium, and their interaction) to test for an overall multivariate treatment effect across the response vector [24,25]. When the MANOVA indicated significant effects, we conducted follow-up two-way ANOVAs for each trait to identify which variables contributed to the multivariate signal [21]. Normality and homoscedasticity were assessed using Shapiro–Wilk and Levene’s tests; when needed, data were transformed prior to inference. Post hoc comparisons were performed using Tukey’s HSD at $\alpha = 0.05$. Given the replication level ($n = 3$ dishes per treatment), results were interpreted with emphasis on effect magnitude and consistency across traits. Analyses were conducted in JMP Pro 16 [28,29]. As a limitation, the design uses three replicates per treatment, which may reduce statistical power for small effects; therefore, non-significant outcomes are interpreted cautiously and discussed in light of biological plausibility and multivariate patterns.

2.6. Software

All analyses were performed in the R statistical computing environment (version 4.2.1) [34]. ANOVA models, Kruskal–Wallis and Wilcoxon tests, and Tukey multiple comparisons were implemented using base R functions. PCA and hierarchical clustering workflows were conducted using FactoMineR (version 2.12) [32], and results extraction and visualization (biplots and dendrograms) were produced with factoextra [33]. Statistical significance was set at $\alpha = 0.05$ for all inferential procedures.

3. Results

3.1. Effect of Fertilization on Germination and Morphometry of *Raphanus sativus* L.

To determine the overall treatment effect, a MANOVA using Pillai's trace was performed. Results showed highly significant differences for both Variety (Pillai = 1.73; $p < 0.001$) and Fertilization (Pillai = 0.80; $p < 0.001$), as well as for their interaction ($V \times F$; Pillai = 1.18; $p < 0.01$).

Univariate ANOVAs confirmed these patterns for most variables, whereas for MGT the nonparametric analysis also indicated significant differences. Variety was the most influential source of variation, particularly for IVG, which showed the highest F value ($F = 169.82$; $p < 0.001$). Importantly, the $V \times F$ interaction was significant across all parameters, demonstrating that the response to fertilization was not uniform but genotype-dependent. Statistical details are summarized in Table 4.

Table 4. Summary of univariate (ANOVA) and multivariate (MANOVA) analyses evaluating genotype and ionic-environment effects.

Source of Variation	DF	MANOVA (Pillai)	ANOVA Germination	ANOVA IVG	ANOVA Root
Variety (V)	2	1.73 ***	$F = 17.05$ ***	$F = 169.82$ ***	$F = 7.87$ ***
Fertilization (F)	1	0.80 ***	$F = 13.47$ **	$F = 35.77$ ***	$F = 12.20$ ***
Interaction ($V \times F$)	2	1.18 **	$F = 9.05$ **	$F = 5.43$ *	$F = 7.12$ **
Residuals	12				

Note. DF: degrees of freedom. Values are Fisher's F statistic, except for MANOVA (Pillai's trace). Significance: $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

Mean comparisons (Table 5) decomposed the interaction patterns. For final germination (%G), varieties AS and GE were stable, with no statistical differences between control and fertilized treatments, maintaining values above 88%. In contrast, ASD showed a severe negative impact under fertilization, dropping from 88.89% (control) to 25.93% (fertilized).

Table 5. Differences in germination and early seedling traits among radish varieties under agar-only (A) and agar + fertilizer (AF) media. Values are means $\pm$ SD ($n = 3$ plates per treatment).

Variety	Fertilization	Germination (%)	MGT (h)	IVG (Index)	Root Length (cm)
AS	A (agar-only)	100.00 ± 0.00^a	40.00 ± 5.33^b	6.22 ± 0.84^a	5.20 ± 2.35^b
	AF (agar + fertilizer)	92.59 ± 6.42^a	49.00 ± 1.73^a	4.44 ± 0.35^b	3.80 ± 1.03^b
ASD	A (agar-only)	88.89 ± 11.11^a	48.89 ± 1.54^a	3.94 ± 0.42^b	9.15 ± 0.22^a
	AF (agar + fertilizer)	25.93 ± 11.11^c	47.50 ± 1.76^a	1.23 ± 0.54^c	5.20 ± 0.76^b
GE	A (agar-only)	96.30 ± 6.42^a	42.06 ± 3.42^b	5.39 ± 0.70^{ab}	9.40 ± 0.49^a
	AF (agar + fertilizer)	88.89 ± 11.11^a	48.11 ± 1.02^a	4.14 ± 0.48^b	4.00 ± 0.81^b

Note. Data are mean $\pm$ SD, with $n = 3$ plates per treatment for germination variables and plate-level root length means (each plate contributed up to 9 seedlings). Different letters within a column indicate significant differences according to the post hoc test used for each variable (Tukey's HSD for %G, IVG, and root length; pairwise Wilcoxon with Benjamini–Hochberg adjustment for MGT; $p < 0.05$).

Regarding speed and development, fertilization tended to delay germination, evidenced by a significant increase in MGT for AS and GE, and a generalized reduction in IVG, with ASD again being the most affected (lowest value in the experiment: 1.23). Root development was strongly inhibited by fertilization in ASD and GE, both showing significant reductions compared to their controls (from 9.15 cm and 9.40 cm down to 5.20 cm and 4.00 cm, respectively). Notably, GE reduced its root length to less than half. By contrast, AS presented the shortest root length in both conditions, with no significant change due to fertilization.

3.2. Association Patterns Among Variables and Treatment Classification

To understand the structure of relationships among variables and to classify treatments based on overall response, a PCA was performed. PCA is explicitly intended for sets of inter-correlated quantitative variables; therefore, correlation among germination indices (e.g., MGT and CVG) does not invalidate the explained-variance percentages, which are derived from the eigen-decomposition of the (standardized) correlation/covariance structure. In this context, highly correlated indices are expected to align with the same latent germination-speed dimension, and interpretation is therefore based on the loading pattern rather than on the number of correlated indicators [46]. Principal component analysis (PCA) revealed that the first two components explained 86.3% of the total variance (PC1 = 62.3%, PC2 = 24%; Figure 4). The biplot indicated that IVG, germination (%), cotyledon width, and cotyledon length contributed most to the separation along PC1, whereas MGT and hypocotyl length loaded more strongly on PC2.

Projection of treatments in the biplot (Figure 4) revealed fertilization-driven dispersion. Variety AS showed high phenotypic stability, with both control and fertilized treatments positioned close to each other and associated with high vigour (positive PC1). In contrast, fertilization produced a pronounced separation in ASD, shifting fertilized samples toward the negative quadrant for both vigour and germination, far from their respective controls.

This pattern was corroborated and formally classified by hierarchical clustering (Figure 5), which identified five groups with distinct behaviors. ASD controls clustered with the best-performing treatments (light-blue cluster), whereas fertilization segregated ASD-Fert into an independent group (red/gray clusters) characterized by low global performance. Additionally, GE (Control) formed an isolated group (dark-blue cluster), clearly differentiated from its fertilized counterpart due to moderate speed and root length.

Finally, Figure 6 shows a differential response in cumulative germination driven by the Variety $\times$ Fertilization interaction. The imported variety GE showed the fastest establishment, reaching near-maximum values by 24 h in medium A (GE_A) and remaining nearly stable up to 72 h, whereas GE_AF began slightly lower but converged to high final percentages. In contrast, domestic materials showed greater medium dependence: AS_A increased markedly between 24 and 48 h and achieved the highest final germination at 72 h, while AS_AF exhibited an initial delay but partially recovered by 72 h. The strongest fertilization effect was observed in ASD, where ASD_AF maintained low germination throughout the assay with higher variability (SE), suggesting an inhibitory effect of mineral supplementation on early emergence in this genotype, unlike ASD_A, which increased rapidly between 24 and 48 h and stabilized thereafter.

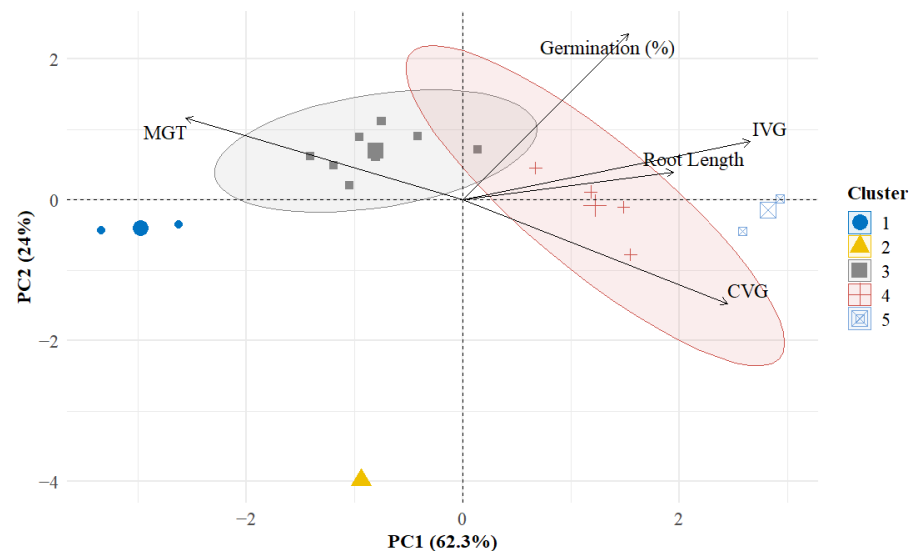


Figure 4. Principal component analysis (PCA) biplot of plate-level responses ($n = 18$; 3 varieties $\times$ 2 media $\times$ 3 replicates) based on standardized germination-performance and early seedling-growth variables: final germination percentage (%G, %), mean germination time (MGT, h), germination speed index (IVG, index), coefficient of velocity of germination (CVG, %), and root length at 72 h (cm). MGT and CVG does not compromise the explained-variance percentages, but indicates shared germination-speed information. Points represent individual plate observations, symbols/colors indicate HCPC cluster membership, and vectors indicate variable loadings in the PCA space. PCA scores are dimensionless because variables were z-standardized before analysis. PC1 and PC2 explained 62.3% and 24.0% of the total variance, respectively. Symbol/color coding indicates HCPC clusters: Cluster 1 (blue circles), Cluster 2 (yellow triangles), Cluster 3 (gray squares), Cluster 4 (red crosses), and Cluster 5 (light-blue squares).

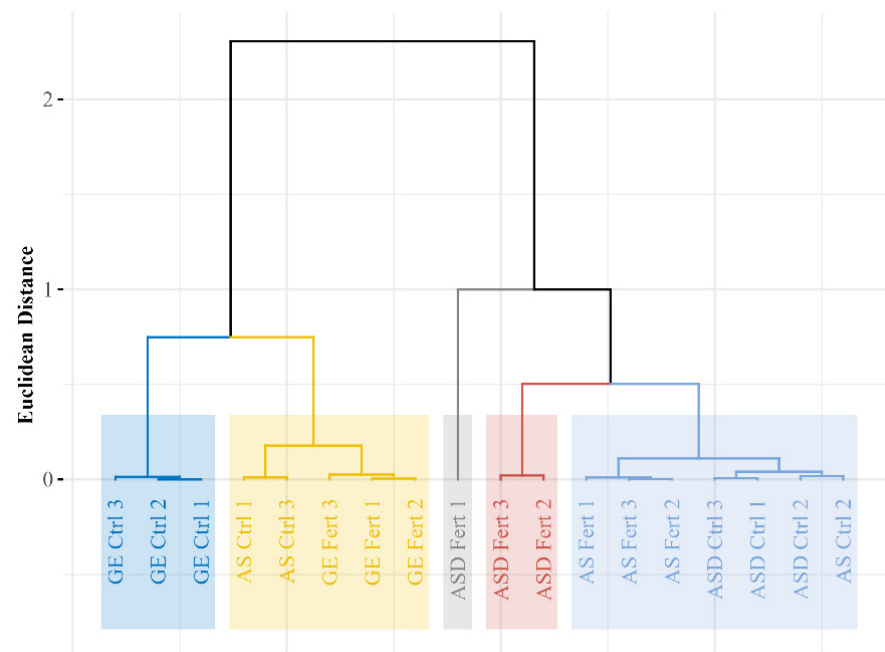


Figure 5. Hierarchical clustering dendrogram of evaluated treatments. Treatment labels follow the format Variety–Medium–Replicate, where AS = Sparkler, ASD = Daikon, and GE = Cherry Belle; A = agar-only and AF = agar + fertilizer; and Replicate denotes the Petri-dish replicate (1–3). Colored background blocks indicate the five clusters identified by hierarchical clustering (dark blue, yellow, gray, red, and light blue).

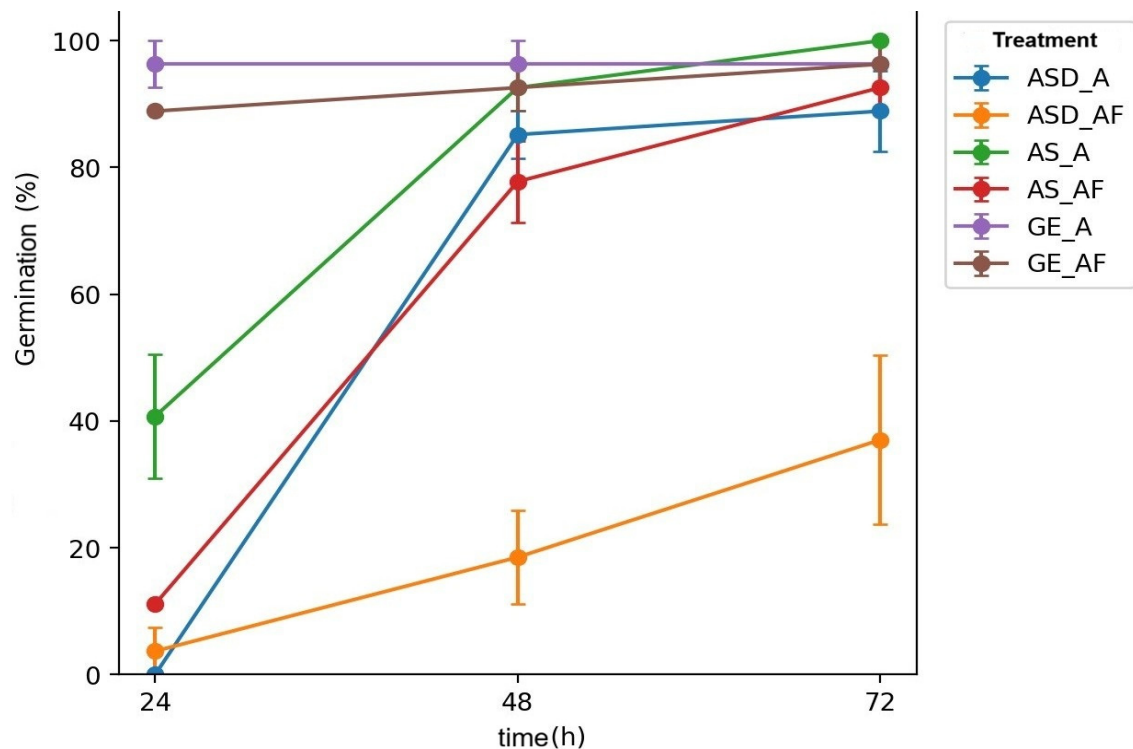


Figure 6. Cumulative germination dynamics by treatment (mean $\pm$ SE).

4. Discussion

The strong Variety $\times$ Fertilization interaction observed in this experiment (notably the collapse of %G and IVG in ASD under AF, together with the marked inhibition of root elongation in ASD and GE) indicates that adding a commercial NPK blend to an agar-based in vitro system acts less as a “universal nutrient boost” and more as a genotype-specific ionic/osmotic filter, where small differences in seed physiology, membrane integrity, reserve mobilization, and ion-handling capacity are amplified under controlled conditions.

A plausible interpretation for the observed delays in germination and reductions in early root length under the fertilizer-supplemented medium is that the AF treatment altered the physicochemical environment of the medium (e.g., solute concentration/osmotic conditions), which may have slowed early emergence and elongation in some genotypes [47–51]. However, because this study measured germination dynamics and plate-level root length only, these results should be interpreted as early phenotypic responses rather than direct evidence of specific physiological mechanisms (e.g., osmotic stress, nutrient toxicity, or improved nutrition). The stronger AF-associated root-length reductions observed in ASD and GE, compared with AS, support a genotype-dependent response to the tested in vitro media conditions [52–54]. Such early-stage sensitivity is consistent with reports that newly emerged seedlings may show delayed growth or reduced vigor under elevated solute concentrations, even when nutrients are present [55].

The fact that GE exhibited rapid establishment in the control yet lost more than half of its root length under AF also fits a stress-response pattern reported in micro-green/brassicaceous systems under combined thermal/ionic constraints highlighting the importance of jointly interpreting germination curves and morphometry instead of relying on %G alone [56]. Statistically, the study’s multi-endpoint structure (several vigor indices + morphology + multivariate profiling) strengthens inference when multiplicity is explicitly controlled, and future expansions with finer temporal scoring or additional traits should consider procedures robust to dependency among tests (common in correlated

vigor variables), such as FDR control under dependency and/or sequentially rejective adjustments to keep conclusions stable as the number of outcomes increases [57,58].

The genotype-dependent patterns observed here are consistent with integrated genotype $\times$ medium effects during early establishment. However, mechanistic explanations involving nitrate signaling, ROS dynamics, or hormonal regulation remain hypotheses in the context of this study, because no physiological or biochemical measurements were collected. Therefore, the present results are best interpreted as a screening-level phenotypic contrast among varieties under two defined in vitro media conditions, with clear differences in germination dynamics and early root elongation [59–71].

Methodologically, these findings support treating commercial fertilizer–agar systems as a low-cost screening tool rather than as a replacement for defined media: classic culture-medium research emphasizes that mineral requirements and ion forms matter, and micropropagation literature shows that “commercially feasible” protocols often succeed by controlling specific medium components and interactions rather than by increasing generic fertilization; thus, a rational next step is to chemically characterize AF (EC, pH, major ion forms, N speciation) and run a small concentration series to identify thresholds where each genotype transitions from stimulation to inhibition [59,60]. In parallel, complementary pre-sowing strategies—such as priming and targeted seed treatments known to improve emergence and early growth under abiotic stress—could be tested as practical countermeasures to AF-induced inhibition, especially for ASD-like sensitive lots [61–63].

Overall, multivariate profiling complemented the univariate results by separating treatments primarily by medium and secondarily by variety (Figure 4), reinforcing the interpretation that medium conditions were a dominant driver of early response patterns [72,73].

5. Conclusions

This study demonstrates that supplementing an agar-based in vitro system with a commercial NPK fertilizer (0.45 g per 200 mL medium; AF treatment) produces a strong genotype-dependent response during early establishment of *Raphanus sativus* L. The significant Variety $\times$ Fertilization interaction confirms that fertilization effects cannot be generalized across materials: while AS and GE maintained high final germination, fertilization tended to delay germination and reduce vigor, and it strongly inhibited root elongation in GE. The national variety ASD exhibited the greatest sensitivity, showing a drastic reduction in germination and the lowest vigor under fertilization, indicating that the fertilizer-generated ionic environment can act as an inhibitory filter for specific genotypes. Multivariate profiling (PCA and hierarchical clustering) integrated germination and growth traits, separating treatments along axes of vigor/architecture and germinative capacity, and consistently classifying treatments into distinct response groups, thereby strengthening interpretation beyond single variables. Overall, a low-cost agar + fertilizer assay is a practical approach to rapidly screen varietal sensitivity to ionic environments, but its use should be supported by dose–response testing and characterization of the medium (e.g., EC/pH and ion forms) to define safe thresholds and improve reproducibility across genotypes and seed lots.

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