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Optimized Water-Agar Assay for Tomato Seed Vigor Enables High-Fidelity Plant Growth Regulator Screening

Mahesh R. Ghule*[1], Monika C. Narayankar[2], Priti B. Panchal[2], Kirti T. Kamthe[3], Adwait K. Kamthe[1] and Sunita S. Sakure[2]

[1]*Green Cultivate Agro Farm, Manjari Bk, Pune, Maharashtra, 412307, India*
[2]*Department of Microbiology, Samaj Bhushan Baburao Alias Appasaheb Jedhe Arts, Commerce & Science College, Pune, Maharashtra, 411002, India*
[3]*Department of Computer Engineering, PDAE College of Engineering, Manjari Bk, Pune, Maharashtra, 412307, India*

Abstract

Tomato (Solanum lycopersicum L.) is a globally important crop where accurate seed quality assessment is essential for uniform crop establishment. Seed vigor, a stronger predictor of field emergence than standard germination percentage, is often inadequately measured by conventional paper towel (PT) assays due to low precision and reproducibility. This study optimized a water–agar (WA) germination assay for tomato (cv. Seminis Abhilash) to provide a reliable platform for vigor evaluation. WA concentrations ranging from 0–2.0% (w/v) were compared with a PT control. While all WA media supported >80% germination by 14 days, the 0.5% WA treatment was optimal, achieving >80% germination within 7 days, with the highest final germination (95%), fastest mean germination time, and maximum vigor index. The optimized WA system was then used to evaluate dose-dependent effects of indole-3-butyric acid (IBA) and gibberellic acid (GA_3), alongside PT. Compared with PT, the WA assay provided greater precision, reflected in lower coefficients of variation, and more clearly resolved hormone dose–response trends. Optimal concentrations were identified at ~50 mg/L IBA and ~100 mg/L GA_3, which maximized seedling vigor. In contrast, the PT method produced variable results that obscured these patterns. Overall, the 0.5% WA assay enhances both accuracy and reproducibility of seed vigor testing and offers strong potential for high-throughput screening of seed treatments and bioactive compounds.

Keywords: *Solanum lycopersicum; seed vigor; water–agar; germination assay; plant growth regulators; high-throughput screening.*

Introduction

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is one of the world's most important vegetable crops, valued for its economic, nutritional, and scientific significance. Originating in western South America, tomato cultivation has expanded globally and today ranks as the second most produced vegetable after potato by volume [1, 2, 3]. In 2022, global tomato production reached ~186.8 million metric tons across 5 million hectares, underpinning a multi-billion-dollar industry that spans fresh produce and processed products such as sauces and pastes [4]. Nutritionally, tomatoes are low in calories but rich in vitamins A, C, and K, minerals, dietary fiber, and phytochemicals, most notably lycopene. Lycopene is a potent antioxidant linked to reduced risks of cardiovascular diseases and several cancers [5, 6, 7, 8]. This combination of agricultural, economic, nutritional, and health value has also made tomato a model organism for studies in genetics, physiology, and biotechnology [1, 9]. Successful and uniform crop establishment begins with seed quality. While viability defines the inherent ability of a seed to germinate, vigor is a superior predictor of performance under diverse and stressful field conditions [10, 11]. Seed vigor is characterized by rapid, uniform germination and robust seedling development. Standard germination tests (SGTs), such as those prescribed by ISTA and AOSA, measure maximum germination potential under optimal conditions [12]. However, these conditions rarely reflect field realities, and SGTs often overestimate emergence capacity. Even weak or damaged seeds may germinate in the laboratory yet fail in the field [10, 13]. To bridge this gap, seed vigor testing has become an essential component of seed quality assessment [14, 15]. For tomato, high vigor is critical for field establishment and yield uniformity [16]. The rolled paper towel (PT) method is widely used for SGTs due to its low cost and simplicity. However, it presents limitations for vigor testing and sensitive applications such as plant growth regulator (PGR) screening. Moisture regulation in PT is inconsistent: excessive wetness can create anaerobic conditions leading to seed rot, while drying can arrest germination. In addition, seedling radicles often entangle within the paper matrix, causing damage during measurement and increasing variability [17, 18]. The opaque, rolled setup further prevents real-time observation of germination. Studies have shown that agar-based substrates generate more uniform seedlings than paper or sand [19]. Water-agar (WA), a sterile, semi-solid, nutrient-free medium, provides several advantages. It offers stable and uniform moisture, prevents substrate entanglement, and allows continuous, non-invasive observation of

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Mahesh R. Ghule | maheshghule2009@gmail.com

germination. However, agar concentration critically influences water potential and gel firmness. Higher agar concentrations reduce water availability by lowering matric potential, imposing resistance to root penetration and creating physiological drought [20, 21]. Conversely, excessively low concentrations may cause free water release or hypoxia [22]. Hydrothermal modeling further confirms that water × temperature interactions strongly regulate germination dynamics [23, 24]. Thus, optimizing agar concentration is essential for accurate assessment of seed vigor.

This study aimed to (1) optimize agar concentration for tomato seed germination and vigor, and (2) compare the optimized WA method with the PT method for high-fidelity screening of tomato seed responses to two PGRs indole-3-butyric acid (IBA) and gibberellic acid (GA_3).

Materials and Methods

Plant Material and Sterilization

Seeds of tomato (*S. lycopersicum* cv. Seminis Abhilash, Bayer Crop Science, India) were used. Seeds were stored at 4 °C in airtight containers. For sterilization, seeds were immersed in 70% ethanol for 1 min, followed by 1.5% sodium hypochlorite with Tween-20 for 10 min, and rinsed five times with sterile distilled water.

Experiment 1: Optimization of WA Concentration

A completely randomized design (CRD) tested six substrates: sterile distilled water (0% agar) and agar concentrations of 0.13, 0.25, 0.50, 1.0, and 2.0% (w/v). Agar (HiMedia, India) was autoclaved (121 °C, 20 min) and poured into sterile 6-well plates (10 mL/well). After solidification, 10 sterilized seeds were placed per well (100 seeds/treatment). For 0% agar, 10 mL sterile water was used. Plates were sealed with Parafilm, incubated at 25 ± 2 °C under a 16 h dark/8 h light regime (ISTA protocol) [12].

Experiment 2: Comparative Analysis of WA vs. PT for PGR Screening

A factorial CRD design compared WA (0.5% agar, from Experiment 1) with PT across six concentrations each of IBA (0, 10, 25, 50, 100, 200 mg/L) and GA_3 (0, 50, 100, 200, 400, 800 mg/L) [25, 26, 27].

WA method: PGRs were filter-sterilized and incorporated into molten 0.5% agar. Ten mL medium was dispensed per well, with 10 seeds/well.

PT method: Two sterile germination papers were moistened with PGR solution, seeds placed, rolled, and enclosed in polyethylene bags.

Each treatment had 10 replicates (100 seeds). Incubation conditions were as in Experiment 1.

Data Collection

In Experiment 1, germination was recorded daily for 14 days. A seed was considered germinated when radicle length ≥2 mm [12]. Final germination %, mean germination time (MGT), shoot/root length, seedling length, seedling vigor index (SVI = germination % × mean seedling length), fresh weight, dry weight, and biomass gain were recorded.

In Experiment 2, germination was assessed on day 7. Shoot and root length, SVI, and qualitative differences between WA and PT seedlings were recorded.

Coefficients of variation (CV) for shoot/root length were calculated.

Statistical Analysis

Data were analyzed by one-way ANOVA (Experiment 1) and two-way ANOVA (Experiment 2) with Tukey's HSD test ($P \leq 0.05$). Data normality and homogeneity were verified; no transformation was required. Analyses were performed using R v4.1.2.

Results and Discussion

Water-Agar Concentration Optimization for Seed Vigor

WA substrates consistently outperformed the water-only control, which was limited by hypoxic stress. The 0.5% WA medium proved optimal, achieving 95% germination and the highest vigor index, consistent with earlier reports on tomato seed vigor under controlled water potential [11,24,29,31]. In the water-only treatment (0% WA), germination was significantly lower and slower than in any agar-containing medium (Table 1). After 14 days, the control reached only ~62% germination, compared with 82–95% across WA treatments.

Seeds germinated in water required considerably more time (MGT ≈ 6.3 days) and produced weak seedlings averaging ~23 mm in total length, which were thin and etiolated. By contrast, even the lowest agar concentration (0.13% WA) supported ~82% germination, with a much faster MGT (~3.5 days) and seedlings averaging ~91 mm in length. These findings highlight the importance of a semi-solid substrate in providing stable moisture and adequate aeration [6, 18, 19].

Among the agar treatments, 0.5% WA emerged as the optimal concentration. Seeds germinated most rapidly at this concentration (MGT ≈ 2.4 days), achieving the highest germination (95%) and producing vigorous seedlings with an average total length of 110 mm and the greatest vigor index (SVI ≈ 9680). Slightly lower or higher agar concentrations were less effective. At 1.0% and 2.0% WA, germination remained relatively high (~84–91%), but root growth was noticeably restricted (average ~39 mm compared with ~50 mm at 0.5%), likely due to reduced water availability and greater resistance to root penetration [20,36]. Conversely, at 0.13% WA, germination was slightly reduced (82%) and growth less uniform, probably because the very soft gel approached a near-liquid state and created localized hypoxic conditions [6].

These results demonstrate that 0.5% WA provides the optimal balance, firm enough to support seed placement and aeration while sufficiently hydrated to promote rapid germination and robust seedling development.

Comparative Performance of WA and PT Methods in PGR Screening

Having established the superiority of 0.5% WA, we compared this system with the standard paper towel (PT) method for screening responses to IBA and GA_3. WA not only enhanced germination and growth but also improved precision in detecting treatment differences [26, 32].

Indole-3-Butyric Acid (IBA)

The WA assay clearly identified an optimal range of 25–50 mg/L, with a decline at higher doses, consistent with auxin's dual roles [30]. At 25 mg/L IBA, WA produced the highest germination (94%) and SVI (~10,800), supported by well-developed roots (~61 mm) and shoots (~52 mm). At 50 mg/L, germination remained high (92%) with similar vigor.

At higher concentrations (100–200 mg/L), performance declined, with germination dropping to ~89–84% and SVI reduced, indicating supra-optimal hormone levels. This biphasic pattern is in line with earlier auxin studies [21, 22].

In contrast, PT controls germinated poorly (~74%), and the best treatment (50 mg/L) reached only ~85% germination and ~7080 SVI. Moreover, PT results showed high variability, often 2–3 times greater standard deviations than WA, obscuring treatment effects. Thus, WA resolved the optimal IBA range (25–50 mg/L) with statistical clarity, whereas PT failed to do so.

Gibberellic Acid (GA_3)

GA_3 promoted germination and shoot elongation across a broad range, with inhibition only at the highest doses. Under WA, concentrations between 50–200 mg/L improved germination and vigor, while 400–800 mg/L caused inhibition. At 100 mg/L GA_3, WA achieved the highest germination (94%) and strong shoot growth (~59 mm), whereas 50 mg/L GA_3 produced the greatest SVI (~10,846) due to balanced shoot and root development. At higher levels (≥400 mg/L), germination and seedling growth declined. These results agree with earlier reports on GA_3-mediated seed germination in tomato [5, 9, 21, 33].

The PT method again showed weaker responses. Although maximal germination in PT (93%) occurred at 100 mg/L GA_3, seedling growth was significantly poorer than WA. For example, WA seedlings at 100 mg/L GA_3 had shoots ~58.6 mm and roots ~51.9 mm, while PT seedlings had shorter shoots (~42.2 mm). Consequently, WA SVI (~10,385) was substantially higher than PT (~9188).

Variability and Statistical Resolution

WA consistently produced lower coefficients of variation (CV %) compared with PT. For example, in IBA trials, WA shoot length CVs ranged from 2.3–3.7%, compared with 2.7–5.3% in PT. Reduced variability allowed finer resolution of treatment differences, such as distinguishing 50 mg/L IBA as superior to 10 mg/L, or identifying 800 mg/L GA_3 as significantly worse than 400 mg/L. Such statistical clarity is crucial for seed vigor assays [1, 3, 19, 26, 28].

Conclusion

The optimized 0.5% WA method enhanced germination, reduced MGT, and produced vigorous seedlings compared with PT. It allowed clear identification of optimal PGR ranges 25–50 mg/L IBA and 50–200 mg/L GA_3 while also revealing inhibitory thresholds at higher concentrations. By combining improved growth outcomes with superior statistical resolution, WA provides a robust platform for seed vigor testing and high-throughput screening of PGRs, biostimulants, and other treatments.

Author Contributions

M.R.G. conceived and designed the experiments. M.R.G., M.C.N., and P.B.P. performed the experiments. M.R.G., A.K.K., and K.T.K. analyzed the data. M.R.G. and K.T.K. wrote the manuscript. S.S.S. reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that they have no conflict of interest.

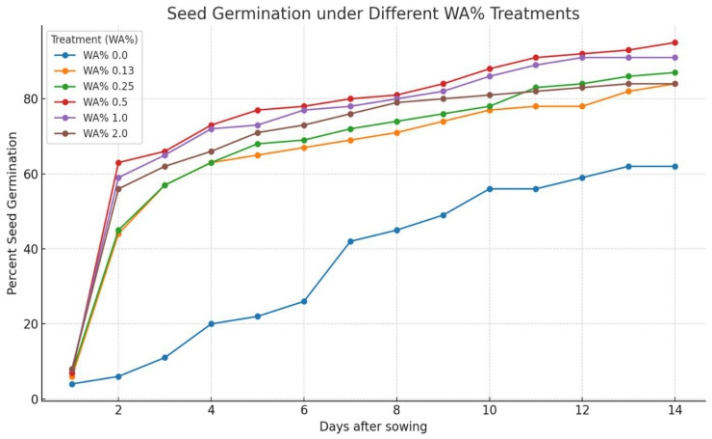

Figure 1. *Tomato seed germination percentage over time (14 days) at different water-agar concentrations. The graph shows germination progress of tomato seeds (Solanum lycopersicum cv. Seminis Abhilash) under six concentrations of water-agar (WA): 0% (water only), 0.13%, 0.25%, 0.5%, 1.0%, and 2.0% (w/v). The non-gelled water control (0% WA) exhibited significantly slower and lower germination compared to any agar-containing medium. Higher WA concentrations, particularly the 0.5% WA, led to the fastest and highest germination rates.*

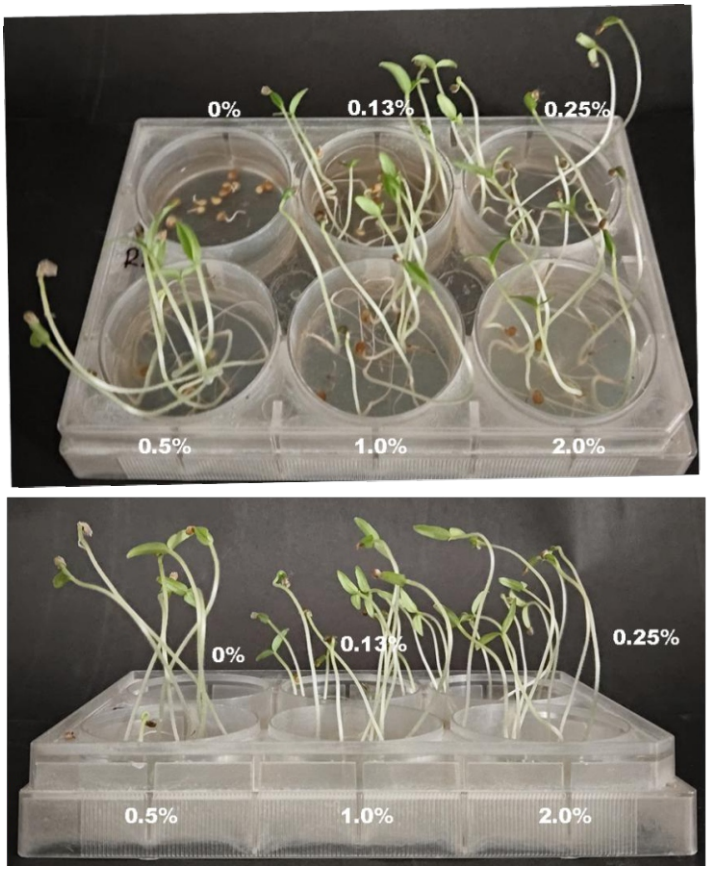

Figure 2. *Effect of water-agar concentration on tomato seedling growth after 14 days. Tomato seeds (Solanum lycopersicum cv. Seminis Abhilash) were germinated for 14 days on media containing 0%, 0.13%, 0.25%, 0.5%, 1.0%, or 2.0% agar (w/v) under controlled conditions (25 ± 2 °C, 12-hour photoperiod). The image shows representative seedlings from each treatment (0% at top left through 2.0% at bottom right). Seedlings grown on 0.5% WA were the largest and most robust, whereas seeds germinated without agar (water only) produced small, spindly seedlings. This visually illustrates the superior seedling vigor achieved at the optimal 0.5% agar concentration.*

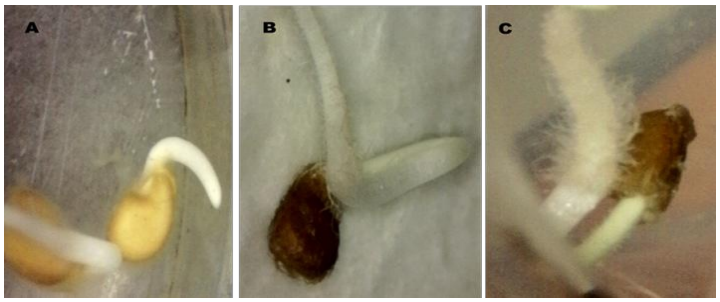

Figure 3. *Radicle morphology of tomato seedlings under different germination substrates (3 days after sowing). Representative images of tomato (Solanum lycopersicum cv. Seminis Abhilash) seedling radicles germinated in:* ***(A)*** *sterile distilled water (0% agar), showing poor radicle elongation and no root hair development;* ***(B)*** *paper towel (PT) method, showing moderate radicle extension and sparse root hair formation due to the semi-humid microenvironment between moist paper layers; and* ***(C)*** *water-agar (WA, 0.5%) method, demonstrating robust radicle growth and profuse root hair formation due to balanced moisture availability and structural support. These observations highlight how the germination substrate influences early root development and seedling vigor. Notably, seedlings grown on WA lifted easily from the medium without root damage, whereas extracting seedlings from PT rolls often resulted in broken root tips or stripped root hairs, underscoring the practical advantages of the WA system for maintaining root integrity and enabling detailed observation of early root growth.*

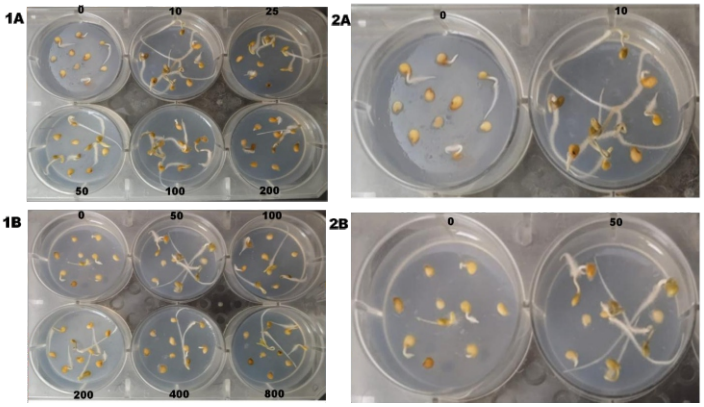

Figure 4*. Dose–response effects of GA_3 and IBA on seed germination of tomato (Solanum lycopersicum cv. Seminis Abhilash) in a six-well plate assay. (1A) Seeds treated with GA_3 at 0, 50, 100, 200, 400, and 800 mg/L. (1B) Seeds treated with IBA at 0, 10, 25, 50, 100, and 200 mg/L. Panels (2A, 2B) illustrate enhanced germination, root elongation, and root hair development at optimal hormone concentrations compared with the untreated control after 2 days.*

Table 1. Effect of water-agar concentration on tomato seed germination and seedling traits (14 days after sowing)

Water Agar (%)	Germination (%)	MGT (days)	Shoot Length (mm)	Root Length (mm)	Total Seedling Length (mm)	Seedling Vigor Index	Fresh Biomass (mg)	Dry Biomass (mg)	Weight Gain (mg)
0.00	62.0 ± 2.0 e	6.29 ± 0.15 e	4.0 ± 1.0 e	19.0 ± 2.0 e	23.0 ± 2.5 e	1288 ± 150 e	78 ± 5 e	18 ± 2 e	60 ± 5 e
0.13	82.0 ± 2.5 cd	3.46 ± 0.12 d	44.0 ± 3.0 cd	47.0 ± 3.5 cd	91.0 ± 4.0 cd	7007 ± 205 cd	184 ± 8 cd	30.2 ± 2.5 cd	153.8 ± 8 cd
0.25	87.0 ± 2.0 bc	3.22 ± 0.10 cd	47.5 ± 3.2 bc	59.0 ± 4.0 b	106.5 ± 4.5 bc	8307 ± 220 bc	195 ± 9 bc	38 ± 3 bc	157 ± 7 bc
0.50	95.0 ± 1.5 a	2.37 ± 0.08 a	60.0 ± 3.5 a	50.0 ± 3.8 bc	110.0 ± 5.0 a	9680 ± 250 a	201 ± 10 a	47 ± 4 a	158.1 ± 6 a
1.00	91.0 ± 1.8 ab	2.56 ± 0.09 ab	58.0 ± 3.0 ab	39.5 ± 3.0 d	97.5 ± 4.2 b	8385 ± 230 b	205 ± 9 a	49 ± 3 a	156 ± 5 bc
2.00	84.0 ± 2.2 de	2.96 ± 0.11 bc	56.0 ± 3.0 b	39.0 ± 3.5 d	95.0 ± 4.0 b	7695 ± 210 cd	191 ± 8 bc	36 ± 3 bc	155 ± 5 c

Values are mean ± SD (n = 10 replicates of 10 seeds each). Within a column, means followed by the same letter are not significantly different (Tukey's HSD, P ≤ 0.05)

Table 2. Comparative effects of Indole-3-butyric Acid (IBA) on tomato seed germination and vigor after 7 days using Water-Agar (WA) and Paper Towel (PT) methods

Method	IBA (mg/L)	Germination (%)	Shoot Length (mm)	Root Length (mm)	Seedling Vigor Index (SVI)
WA	0	81.01 ± 0.70 e	47.27 ± 1.27 d	54.20 ± 1.32 c	8878.63 ± 224.88 d
	10	83.20 ± 0.33 d	47.44 ± 1.61 c	62.50 ± 1.30 a	7987.75 ± 232.39 e
	25	94.22 ± 0.25 a	52.04 ± 1.21 a	61.18 ± 1.17 a	10869.12 ± 228.48 a
	50	92.21 ± 0.24 b	49.19 ± 1.28 b	59.09 ± 0.79 b	9961.76 ± 125.16 b
	100	89.24 ± 0.56 c	47.51 ± 1.73 c	53.39 ± 1.65 d	9181.90 ± 264.81 c
	200	84.39 ± 0.22 d	46.50 ± 1.70 e	43.66 ± 1.09 e	7573.44 ± 245.28 g
PT	0	74.03 ± 0.45k	28.04 ± 0.77 l	42.94 ± 1.54 i	5252.52 ± 162.80 l
	10	79.99 ± 0.38 i	36.58 ± 0.98 i	44.58 ± 1.61 g	6484.98 ± 208.62 i
	25	81.65 ± 0.18 h	37.56 ± 1.24 h	45.88 ± 0.53 e	6802.11 ± 145.35 h
	50	84.57 ± 0.75 f	38.12 ± 2.02 g	45.76 ± 1.34 f	7079.07 ± 285.39 f
	100	76.74 ± 0.34 j	35.99 ± 1.43 j	42.71 ± 1.38 j	6044.16 ± 214.27 j
	200	72.70 ± 0.61 l	33.80 ± 1.23 k	40.52 ± 1.84 l	5351.04 ± 195.12 k

Data are mean ± SD. Means within the entire table followed by the same letter are not significantly different (Tukey's HSD, P≤0.05)

Table 3. Comparative Effects of Gibberellic Acid (GA_3) on Tomato Germination and Vigor Using Water-Agar (WA) and Paper Towel (PT) Methods

Method	GA_3 (mg/L)	Germination (%)	Shoot Length (mm)	Root Length (mm)	Seedling Vigor Index (SVI)
WA	0	80.77 ± 0.47 h	44.13 ± 0.43 e	43.56 ± 0.15 j	7716.72 ± 51.04 h
	50	88.11 ± 0.40 a	59.41 ± 0.42 a	51.27 ± 0.30 h	10846.64 ± 69.58 a
	100	94.14 ± 0.18 b	58.55 ± 0.49 b	51.93 ± 0.29 g	10385.12 ± 72.38 b
	200	89.05 ± 0.38 g	48.76 ± 0.67 d	51.00 ± 0.28 i	8878.44 ± 84.54 e
	400	81.02 ± 0.55 e	55.46 ± 0.52 c	52.61 ± 0.38 e	9834.37 ± 82.81 c
	800	77.35 ± 0.29 k	40.80 ± 0.30 h	39.07 ± 0.36 l	6150.00 ± 51.60 k
PT	0	74.42 ± 0.31 l	27.48 ± 0.26 l	41.83 ± 0.35 k	5128.94 ± 44.40 l
	50	89.82 ± 0.63 f	37.93 ± 0.33 i	53.92 ± 0.28 c	8266.50 ± 54.90 g
	100	93.20 ± 0.23 c	42.19 ± 0.39 f	56.61 ± 0.34 a	9188.40 ± 67.89 d
	200	91.11 ± 0.10 d	41.21 ± 0.40 g	55.85 ± 0.42 b	8832.46 ± 75.54 f
	400	83.52 ± 0.42 i	37.49 ± 0.55 j	53.22 ± 0.27 d	7528.93 ± 67.24 i
	800	81.06 ± 0.66 j	35.18 ± 0.40 k	52.18 ± 0.36 f	7076.16 ± 63.99j

Data are mean ± SD. Means within the entire table followed by the same letter are not significantly different (Turkey's HSD, P≤0.05)

Table 4. Coefficient of variation (CV %) for seedling length measurements under different PGR treatments, comparing the Water-Agar (WA) and Paper Towel (PT) methods

PGR	Conc. (mg/L)	Method	CV Shoot Length (%)	CV Root Length (%)
IBA	0	WA	2.7	2.4
		PT	2.8	3.6
	10	WA	3.4	3.1
		PT	4.0	3.2
	25	WA	3.6	3.1
		PT	2.7	3.6
	50	WA	2.3	1.9
		PT	3.3	1.2
	100	WA	2.6	1.3
		PT	5.3	3.0
	200	WA	3.7	2.5
		PT	3.6	4.5
GA_3	0	WA	1.0	0.3
		PT	0.9	0.8
	50	WA	0.9	0.7
		PT	1.5	0.5
	100	WA	0.7	0.6
		PT	1.0	0.8
	200	WA	0.8	0.6
		PT	0.9	0.6
	400	WA	1.4	0.5
		PT	0.9	0.5
	800	WA	0.7	0.9
		PT	1.1	0.7

Across most treatments, the WA method yielded equal or lower variability than the PT method, indicating more uniform growth and measurement conditions.

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