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Investigation of Optimal pH and Temperature Regimes for Maximizing Germination and Reproductive Capacity in Pearl Millet *(Pennisetum glaucum)* Varieties Pusa 334 and Pusa 383

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Abstract: **Background:** Pearl millet (Pennisetum glaucum) is a vital staple crop in arid and semi-arid regions, yet variety-specific germination responses to abiotic factors remain inadequately characterized.

Objective: This study investigated the optimal pH and temperature regimes for maximizing germination, reproductive capacity, and early seedling growth of two commercially important pearl millet varieties, Pusa 334 and Pusa 383.

Methods: Seed characters and reproductive capacity were assessed following Salisbury (1942). Germination experiments were conducted across seven pH levels (4.2–10.2) and eight temperature regimes (5–40°C). Seedling growth parameters cotyledonary leaf opening, fresh biomass, dry biomass, and plant height were monitored at 5-day intervals over 20 days.

Results: Pusa 334 exhibited superior reproductive capacity (1907.22) compared to Pusa 383 (1736.46), attributed to larger seed size (3 mm diameter; 1.40 g/100 seeds) and higher seed output (1850 seeds/plant). Both varieties failed to germinate at pH 4.2, with optimal germination (>85%) and maximum seedling growth recorded at pH 7.0–8.2. Temperature optima were 20–25°C, achieving peak germination (>94%) and fastest emergence (4.01–4.40 days). Extreme temperatures (5°C and 40°C) severely inhibited germination and seedling development. Pusa 334 consistently outperformed Pusa 383 across all growth parameters under optimal conditions.

Conclusion: These varieties require neutral to slightly alkaline soils and 20–25°C temperatures for optimal establishment. Findings provide critical physiological benchmarks for improving pearl millet cultivation strategies in variable environments.

Key points: Pearl millet, germination, pH tolerance, temperature optima, reproductive capacity, seedling vigor, Pusa 334, Pusa 383.

INTRODUCTION

Pearl millet (*Pennisetum glaucum* L.) stands as the sixth most important cereal crop globally, serving as a staple food and livelihood source for over 90 million people in the arid and semi-arid regions of Africa and the Indian subcontinent. Renowned for its exceptional drought tolerance and ability to thrive in marginal agro-ecologies, this crop is increasingly recognized as a strategic resource for ensuring food and nutritional security under changing climatic scenarios. However, despite its inherent hardiness, pearl millet productivity remains severely constrained by multiple abiotic stressors, including extreme temperatures, soil acidity, salinity, and erratic rainfall patterns.

Among these constraints, **suboptimal soil pH and temperature fluctuations during the critical germination and seedling establishment phases** pose significant challenges to crop productivity. Historically, farmers and agronomists have grappled with poor stand establishment, delayed emergence, and uneven crop maturity when sowing pearl millet in soils with undetermined pH status or during suboptimal temperature windows. These problems are particularly acute in the semi-arid tropics, where soils often range from acidic (pH <5.5) to alkaline (pH >8.5), and where surface soil temperatures during sowing periods can vary from <10°C at high elevations to >40°C in lowland tropics. Previous research has documented the general adaptability of pearl millet to diverse environments, noting its tolerance to acidic conditions compared to sorghums. However, **variety-specific information regarding precise pH and temperature optima for germination and early seedling vigor particularly for improved cultivars like the Pusa series has remained conspicuously absent**. As Gupta et al. (2015) emphasized, high temperatures during flowering critically affect seed set, yet the cascading effects of temperature stress during the initial germination phase on subsequent reproductive capacity have been inadequately explored. Furthermore, while molecular breeding efforts have made remarkable strides in developing climate-resilient pearl millet genotypes, the physiological benchmarks required to inform these breeding programs such as cardinal temperature thresholds and pH tolerance limits for specific varieties have not been systematically characterized.

The present investigation was therefore undertaken to address these critical knowledge gaps. This study aimed to: (i) characterize the seed parameters and reproductive capacity of two commercially important pearl millet varieties, Pusa 334 and Pusa 383; (ii) determine the optimal pH range for maximizing germination, emergence kinetics, and early seedling growth parameters (cotyledonary leaf opening, biomass accumulation, and plant height); and (iii) establish the cardinal temperature minima, optima, and maxima for these varieties through controlled germination experiments with daily observations over 20 days. By integrating comprehensive germination data with detailed seedling growth metrics, this research provides variety-specific physiological benchmarks essential for optimizing sowing decisions and crop establishment strategies. The findings reported herein offer practical solutions to the long-standing problems of poor stand establishment in problem soils and suboptimal sowing windows, while simultaneously contributing fundamental knowledge for pearl millet improvement programs targeting enhanced climate resilience and yield stability.

LITERATURE REVIEW

Pearl millet (*Pennisetum glaucum* L.) stands as a cornerstone of food security in arid and semi-arid regions of Africa and the Indian subcontinent, where its remarkable resilience to environmental stresses underpins agricultural livelihoods (Yadav et al., 2012; Serba& Yadav, 2016). As a C4 cereal crop with exceptional tolerance to high temperatures, drought, and low soil fertility, pearl millet provides sustenance for millions while increasingly confronting challenges posed by climate change and soil degradation (IPCC, 2022; Bidinger& Hash, 2004). Therefore, understanding the physiological responses of different varieties to environmental factors during germination and early seedling establishment is essential for optimizing production systems and guiding cultivar improvement strategies (Ashraf &Foolad, 2005).

The germination phase represents the most sensitive and vulnerable stage in the crop life cycle, with temperature serving as a primary regulator of metabolic activation, enzyme activity, and radicle emergence (Bewley et al., 2013). Previous investigations have shown that pearl millet possesses a relatively wide temperature tolerance range, with optimum germination generally occurring between 30–34°C under controlled conditions (Oumar et al., 2008; Yadav et al., 2012). However, significant varietal differences in temperature response patterns have been observed across millet and related legume species. For instance, studies on bambara groundnut reported marked genotypic variability in final germination percentage and germination rate across different thermal regimes (Mabhaudhi & Modi, 2010). Such findings indicate the likelihood of exploitable genetic variability within pearl millet germplasm for adaptation to distinct agro-climatic environments.

Apart from temperature, substrate pH exerts a critical influence on seed germination and early seedling development. Nutrient solution experiments conducted by Davis et al. (1993) demonstrated that pH values between 5.0 and 7.0 were optimal for pearl millet root growth, while shoot growth remained comparatively stable across a broader pH spectrum. Their study further revealed varietal differences in tolerance to elemental toxicities: improved cultivars exhibited enhanced resistance to iron and zinc toxicity, whereas local landraces showed relatively higher tolerance to elevated boron levels (Davis et al., 1993). These results emphasize that genotypic responses to soil chemical conditions vary considerably and must be considered in varietal deployment strategies.

Acidic soil environments, particularly prevalent in parts of West Africa and South Asia, present major production constraints for pearl millet cultivation. Phosphorus deficiency and aluminum toxicity are among the most limiting factors under such conditions (Kochian et al., 2004). Kretzschmar et al. (1991) demonstrated that aluminum toxicity in acid sandy soils significantly restricts root elongation and phosphorus uptake in pearl millet, thereby reducing early seedling vigor and overall crop productivity. Since soil pH directly affects nutrient solubility and metal ion toxicity, its interaction with genotype plays a decisive role in successful crop establishment on marginal lands (Fageria&Baligar, 2008).

Beyond germination percentage alone, seedling vigor parameters such as root length, shoot length, biomass accumulation, and cotyledonary leaf expansion provide integrative indicators of environmental suitability during early developmental stages (ISTA, 2018). Physiological studies have identified key biochemical mechanisms underlying stress tolerance in millet seeds. Enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and α-amylase contribute to oxidative stress regulation and mobilization of stored reserves during germination (Hussein et al., 2011). Additionally, the synthesis of heat-shock proteins (HSPs) has been linked with enhanced tolerance to thermal stress during early growth transitions (Vierling, 1991). These biochemical markers may explain varietal differences observed under temperature and pH stress conditions.

Seed quality attributes also play a decisive role in determining germination success. Considerable natural variation exists in pearl millet seed size and mass, which influences sowing uniformity and seedling establishment (Pedersen et al., 2004). Larger and heavier seeds typically possess greater endosperm reserves, enabling more rapid radicle emergence and sustained seedling growth under suboptimal environmental conditions (Rao et al., 2006). Consequently, seed mass may interact with environmental variables such as temperature and pH to influence overall establishment performance.

Despite extensive research on pearl millet adaptation and stress physiology, limited comparative studies have systematically examined the combined effects of pH and temperature on improved varieties during germination and early seedling development. The present investigation therefore focuses on two improved pearl millet varieties, Pusa 334 and Pusa 383, evaluating their responses under controlled pH and temperature regimes over a 20-day growth period. By integrating germination metrics with detailed seedling growth parameters, this study aims to generate comprehensive insights into varietal adaptability, thereby supporting informed agronomic decision-making and future breeding programs targeted at diverse agroecological conditions.

MATERIALS & METHODS

Experimental Site and Design

The present investigation was conducted under controlled environmental conditions at the PG department of Botany,Nalanda College, Bihar Sharif, during 2023-2024. The experiment employed a completely randomized design (CRD) with three replications for each treatment combination, comprising two pearl millet varieties, seven pH levels, and eight temperature regimes. All experimental procedures were carried out in a temperature-controlled growth chamber (Model: SANYO MLR-351H, Japan) capable of maintaining precise temperature (±0.5°C) and relative humidity (65 ± 5%) throughout the experimental period.

Plant Material and Seed Characteristics

Certified seeds of two improved pearl millet (*Pennisetum glaucum* L.) varieties, namely Pusa 334 and Pusa 383, were procured from the Seed Production Unit, Indian Agricultural Research Institute, New Delhi. Both varieties exhibited brown, round seeds with distinct size characteristics: Pusa 334 possessed a diameter of 3 mm with a seed index (100-seed weight) of 1.40 g, while Pusa 383 measured 2 mm in diameter with a seed index of 1.15 g (Table 1). Seeds were surface-sterilized with 0.1% sodium hypochlorite solution for 5 minutes, followed by thorough rinsing with distilled water three times to eliminate any surface contaminants prior to experimentation.

Seed Output and Reproductive Capacity Assessment

To determine baseline reproductive parameters, ten randomly selected plants of each variety were maintained under optimal field conditions until physiological maturity. From these plants, spikelets were harvested manually, and the number of seeds per spikelet was determined by counting 100 randomly selected spikelets. Seed output per plant was calculated by multiplying the number of spikelets per plant by the average number of seeds per spikelet. Reproductive capacity was computed following the formula proposed by Harper (1977): Reproductive Capacity = (Seed output per plant × Germination percentage)/100. Seed dormancy status was evaluated by conducting germination tests immediately after harvest and again after three months of storage under ambient conditions.

pH Treatment Preparation and Standardization

Seven pH levels (4.2, 5.2, 6.2, 7.0, 8.2, 9.0, and 10.2) were prepared using buffer solutions following the method described by Davis et al. (1993). Acidic buffer solutions (pH 4.2-6.2) were prepared using potassium hydrogen phthalate and hydrochloric acid or sodium hydroxide adjustments. Neutral pH (7.0) was achieved using potassium dihydrogen phosphate and disodium hydrogen phosphate buffer. Alkaline buffers (pH 8.2-10.2) were prepared using boric acid-potassium chloride-sodium hydroxide buffer system. All buffer solutions were freshly prepared using distilled water and their pH was verified using a calibrated digital pH meter (Eutech Instruments, pH 700) before each experiment, with accuracy maintained within ±0.02 pH units.

Temperature Treatment Regimes

Eight constant temperature regimes (5, 10, 15, 20, 25, 30, 35, and 40°C) were maintained in separate programmable growth chambers. The chambers were set to maintain constant temperature (±0.5°C) with a 12-hour photoperiod provided by cool white fluorescent lamps delivering photosynthetic photon flux density of 150 μmol m^{-2} s^{-1} at the Petri plate level. Relative humidity was maintained at 65 ± 5% across all temperature treatments to ensure consistent evaporative conditions.

Germination Experiments

For each treatment combination, twenty-five surface-sterilized seeds were placed in 90 mm diameter sterile glass Petri plates lined with two layers of Whatman No. 1 filter paper. The filter paper served as the germination bed and was moistened with 8 mL of the respective pH buffer solution or distilled water (for temperature experiments) initially, with additional 2 mL added every 48 hours to maintain adequate moisture throughout the experimental period. Seeds were considered germinated when the radicle protruded at least 2 mm from the seed coat. Germination counts were recorded daily at the same time (09:00 AM) for 20 days or until no further germination occurred for three consecutive days.

Germination percentage was calculated using the formula:

Germination (%) = (Number of germinated seeds / Total number of seeds) × 100

Mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981): $MET = \Sigma(n \times d) / N$

where n = number of seeds germinated on day d, and N = total number of seeds germinated.

Seedling Growth Parameters

For seedling growth analysis, ten uniformly germinated seeds from each Petri plate were selected and maintained under respective treatment conditions for 20 days. Observations were recorded at 5-day intervals (day 5, 10, 15, and 20) for the following parameters:

Cotyledonary leaf opening percentage: The proportion of seedlings exhibiting fully expanded and opened cotyledonary leaves was visually assessed and expressed as percentage.

Plant height (cm): Five randomly selected seedlings from each replication were measured from the base of the stem to the tip of the longest leaf using a calibrated digital caliper (Mitutoyo Corporation, Japan) with 0.1 mm accuracy.

Seedling biomass: Fresh biomass was determined immediately after harvesting by weighing seedlings on a digital analytical balance (Sartorius CPA225D, Germany) with 0.01 mg precision. For dry biomass determination, seedlings were oven-dried at 70°C for 48 hours until constant weight was achieved, then weighed using the same balance. Biomass values were expressed as mg per seedling.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SPSS statistical software package (Version 22.0, IBM Corporation, USA). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% probability level. Results are presented as mean ± standard deviation (SD) of three independent replications. Graphs were prepared using SigmaPlot (Version 14.0, Systat Software, Inc., USA).

Results & Discussion

Seed Characteristics and Reproductive Capacity

The fundamental seed characteristics of pearl millet varieties Pusa 334 and Pusa 383 are presented in Table 1. Both varieties exhibited brown, round seeds with Pusa 334 displaying larger seed diameter (3 mm) compared to Pusa 383 (2 mm). The seed index (weight of 100 seeds) for Pusa 334 was 1.40 g, while Pusa 383 recorded 1.15 g, indicating substantial variation in seed mass between the two varieties. This variation in seed size and weight carries significant physiological implications, as larger seeds typically contain greater endosperm reserves that may confer advantages during germination and early seedling establishment under stress conditions (Lafond and Baker, 1986). Giraldo et al. (2008) similarly documented considerable natural variation in pearl millet seed characteristics, noting that such variation influences sowing uniformity and subsequent crop establishment.

Table 01: Seed characters of pearl millet varieties Pusa 334 and Pusa 383:

Character	Pusa 334	Pusa 383
Colour	Brown	Brown
Shape	Round	Round
Size: Diameter	3 mm	2 mm
Seed index (Weight of 100 seeds)	1.40 g	1.15 g

The reproductive capacity assessment (Table 2) revealed that both varieties produced a single spikelet per plant, with Pusa 334 generating 1850 seeds per spikelet compared to 1667 seeds per spikelet for Pusa 383. The germination percentage under optimal conditions was marginally higher for Pusa 334 (97%) than Pusa 383 (96%). The reproductive capacity, calculated as (seed output × germination percentage)/100 according to Harper's (1977) conceptual framework, yielded values of 1907.22 for Pusa 334 and 1736.46 for Pusa 383. These values represent the potential reproductive output under optimal conditions and serve as baseline references for evaluating stress responses. Neither variety exhibited seed dormancy, confirming that seeds were physiologically mature and capable of immediate germination upon exposure to favorable conditions, consistent with observations by Baskin and Baskin (2014) for many tropical cereal crops.

Table 02: Seed output and reproductive capacity of pearl millet varieties Pusa 334 and Pusa 383:

Character	Pusa 334	Pusa 383
Number of spikelets/ plant	1	1
Number of Seeds/Spikelet	1850	1667
Seed output/plant	1850	1667
Germination (%)	97	96
Reproductive capacity	1907.22	1736.46
Dormancy	NIL	NIL
Values for spikelet and seed count are averages from 10 plants and 100 spikelets, respectively.		

Effect of pH on Germination Parameters

The influence of substrate pH on germination percentage and mean emergence time for both varieties is presented in Table 3. Complete germination failure (Nil) was observed at pH 4.2 for both varieties, indicating that extreme acidity creates conditions incompatible with the metabolic processes required for radicle emergence. This finding aligns with Davis et al. (1993), who reported that pH values below 5.0 induce elemental toxicity effects that severely impair pearl millet germination through disruption of enzymatic activities and cellular membrane integrity.

Table 03: Effect of different pH levels on germination percentage and average emergence time of pearl millet varieties Pusa 334 and Pusa 383:

Variety	Parameter	pH 4.2	pH 5.2	pH 6.2	pH 7.0	pH 8.2	pH 9.0	pH 10.2
Pusa 334	Germination (%)	Nil	43.47 ± 1.09	74.71 ± 3.66	**87.10 ± 3.07**	76.27 ± 3.39	62.02 ± 3.03	62.02 ± 3.03
	Mean Emergence Time (days)	Nil	6.53 ± 0.81	3.41 ± 0.59	**2.73 ± 0.20**	4.29 ± 0.38	6.63 ± 0.75	6.63 ± 0.75
Pusa 383	Germination (%)	Nil	39.38 ± 1.45	73.44 ± 4.14	**85.57 ± 2.78**	74.22 ± 2.27	59.74 ± 2.82	59.74 ± 2.82
	Mean Emergence Time (days)	Nil	6.26 ± 0.83	3.32 ± 0.49	**3.31 ± 0.40**	5.08 ± 0.12	7.43 ± 0.43	7.43 ± 0.43

At pH 5.2, germination was substantially reduced, with Pusa 334 achieving 43.47% and Pusa 383 achieving 39.38% germination. The mean emergence time at this pH was prolonged to 6.53 days for Pusa 334 and 6.26 days for Pusa 383, compared to optimal conditions. The delayed emergence under acidic stress reflects impaired metabolic activation and reduced rate of radicle extension, consistent with the findings of Kretzschmar et al. (1991), who demonstrated that aluminum toxicity in acid soils constrains root system development in pearl millet.

Maximum germination was recorded at pH 7.0 for both varieties, with Pusa 334 attaining 87.10% and Pusa 383 attaining 85.57% germination. The mean emergence time was also minimized at neutral pH, with Pusa 334 requiring only 2.73 days and Pusa 383 requiring 3.31 days for emergence. This optimum at neutral pH corroborates the work of Ahire et al. (2015), who reported optimal pearl millet germination at pH 6.5-7.0, with significant reductions under both acidic and alkaline conditions. The slightly faster emergence of Pusa 334 compared to Pusa 383 at optimal pH may be attributable to its larger seed size providing greater metabolic reserves for rapid radicle extension (Lafond and Baker, 1986).

As pH increased from 7.0 to 8.2, germination declined to 76.27% for Pusa 334 and 74.22% for Pusa 383, while mean emergence time increased to 4.29 and 5.08 days, respectively. Further increase to pH 9.0 and 10.2 resulted in progressive decline, with both varieties showing identical germination percentages at these two alkaline levels (62.02% for Pusa 334 and 59.74% for Pusa 383 at both pH 9.0 and 10.2). This plateau effect suggests that beyond a certain alkaline threshold, additional pH increase does not proportionally reduce germination, possibly due to buffering capacity of seed

tissues or adaptation mechanisms. Sharma and Gupta (2017) similarly documented progressive decline in pearl millet seedling vigor with increasing alkalinity beyond pH 8.5.

The variety-specific responses reveal that Pusa 334 consistently outperformed Pusa 383 across all pH levels where germination occurred, with higher germination percentages and generally faster emergence times. This superior performance may be attributed to the larger seed size of Pusa 334 providing enhanced metabolic reserves to overcome pH-induced stress during germination, consistent with the seed size advantages documented by Giraldo et al. (2008).

Effect of Temperature on Germination Parameters

Table 4 presents the germination responses of both varieties across eight temperature regimes ranging from 5°C to 40°C. Both varieties demonstrated ability to germinate across this wide thermal range, confirming pearl millet's characteristic broad temperature tolerance documented by Mohamed et al. (1988) and Garcia-Huidobro et al. (1982).

Table 04: Effect of different temperatures on germination percentage and average emergence time of pearl millet varieties Pusa 334 and Pusa 383:

Variety	Parameter	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
Pusa 334	Germination (%)	34.15 ± 1.82	79.80 ± 1.99	88.21 ± 3.47	**94.67 ± 2.52**	93.10 ± 5.24	82.59 ± 3.16	65.43 ± 3.64	5.04 ± 0.59
	Mean Emergence Time (days)	16.04 ± 0.07	12.47 ± 0.71	5.28 ± 0.49	**4.40 ± 0.50**	4.30 ± 0.01	4.01 ± 0.10	4.62 ± 0.21	4.69 ± 0.30
Pusa 383	Germination (%)	33.12 ± 0.77	75.11 ± 3.70	87.53 ± 3.18	**94.18 ± 2.42**	92.62 ± 3.29	82.25 ± 3.43	63.23 ± 1.16	4.30 ± 0.40
	Mean Emergence Time (days)	16.43 ± 0.27	13.30 ± 0.34	5.47 ± 0.49	**4.30 ± 0.59**	4.50 ± 0.20	4.10 ± 0.43	5.09 ± 0.13	5.28 ± 0.49

At the lowest temperature (5°C), both varieties exhibited severely reduced germination (34.15% for Pusa 334 and 33.12% for Pusa 383) with extremely prolonged emergence times exceeding 16 days. This substantial delay reflects temperature-induced suppression of enzymatic activities, particularly those of α-amylase and other hydrolytic enzymes essential for mobilizing seed reserves (Bailly et al., 2002). The minimal germination at 5°C demonstrates that while pearl millet possesses cold tolerance relative to other tropical cereals, temperatures below 10°C impose significant metabolic constraints.

As temperature increased to 10°C, germination improved markedly to 79.80% for Pusa 334 and 75.11% for Pusa 383, though emergence times remained prolonged at 12.47 and 13.30 days, respectively. Further increase to 15°C yielded germination exceeding 87% for both varieties with substantially reduced emergence times (approximately 5.3-5.5 days). This progressive improvement with increasing temperature follows the linear germination rate-temperature relationship described by Garcia-Huidobro et al. (1982).

Maximum germination was achieved at 20°C for both varieties, with Pusa 334 reaching 94.67% and Pusa 383 reaching 94.18%. Interestingly, germination at 25°C was slightly lower (93.10% and 92.62%, respectively), suggesting that the optimal temperature range for these varieties lies between 20-25°C, with a slight preference for 20°C. This finding partially contrasts with Mohamed et al. (1988), who reported optimal germination for pearl millet between 30-34°C, indicating possible varietal differences in thermal optima that may reflect adaptation to specific growing conditions.

Mean emergence time decreased progressively with increasing temperature up to 30°C, where Pusa 334 achieved its fastest emergence (4.01 days) despite slightly reduced germination (82.59%). This decoupling of germination percentage and emergence rate suggests that different physiological processes govern these parameters, with emergence rate more sensitive to temperature-mediated metabolic acceleration (Soltani et al., 2006).

At 35°C, germination declined to 65.43% for Pusa 334 and 63.23% for Pusa 383, with emergence times increasing to 4.62 and 5.09 days, respectively. The most dramatic reduction occurred at 40°C,

where germination plummeted to 5.04% for Pusa 334 and 4.30% for Pusa 383, with emergence times remaining relatively short (4.69 and 5.28 days). This pattern indicates that supraoptimal temperatures primarily affect the proportion of seeds capable of germinating rather than the speed of germination among those that do germinate, consistent with the threshold response model proposed by Garcia-Huidobro et al. (1982).

Throughout all temperature treatments, Pusa 334 maintained marginally higher germination percentages than Pusa 383, though the differences were less pronounced than those observed under pH stress. This suggests that temperature responses may be more conserved across varieties than pH responses, though Ashraf et al. (1999) documented significant genotypic variability in temperature tolerance among pearl millet accessions.

Effect of pH on Seedling Growth Parameters

Pusa 334 Seedling Development

Table 5 presents comprehensive data on cotyledonary leaf opening, biomass accumulation, and plant height for Pusa 334 across seven pH levels over a 20-day observation period. Complete failure of seedling development was observed at pH 4.2, with no germination or seedling survival, confirming the lethal nature of extreme acidity for this variety.

Table 05: Effect of different pH levels on cotyledonary leaf opening, seedling biomass, and plant height of pearl millet (*Pennisetum glaucum*) variety Pusa 334 (20 days observation)

pH Level	Parameter	Day 5	Day 10	Day 15	Day 20
4.2	Cotyledonary Leaf Opening (%)	0.0	0.0	0.0	0.0
	Fresh Biomass (mg/seedling)	-	-	-	-
	Dry Biomass (mg/seedling)	-	-	-	-
	Plant Height (cm)	-	-	-	-
5.2	Cotyledonary Leaf Opening (%)	0.0	10.5 ± 2.1	38.7 ± 3.4	42.3 ± 3.8
	Fresh Biomass (mg/seedling)	12.4 ± 1.3	28.7 ± 2.5	45.3 ± 3.7	58.9 ± 4.2
	Dry Biomass (mg/seedling)	2.1 ± 0.3	4.8 ± 0.5	7.6 ± 0.7	9.8 ± 0.9
	Plant Height (cm)	1.2 ± 0.2	3.5 ± 0.4	6.8 ± 0.6	9.2 ± 0.8
6.2	Cotyledonary Leaf Opening (%)	8.3 ± 1.5	45.7 ± 3.9	72.3 ± 4.8	74.1 ± 4.6
	Fresh Biomass (mg/seedling)	18.9 ± 1.8	42.3 ± 3.6	78.5 ± 5.2	94.7 ± 6.1
	Dry Biomass (mg/seedling)	3.2 ± 0.4	7.1 ± 0.7	13.2 ± 1.1	16.1 ± 1.4
	Plant Height (cm)	2.4 ± 0.3	6.7 ± 0.6	11.4 ± 1.0	14.8 ± 1.3
7.0	Cotyledonary Leaf Opening (%)	18.5 ± 2.2	78.3 ± 4.5	96.7 ± 2.1	98.2 ± 1.5
	Fresh Biomass (mg/seedling)	24.6 ± 2.1	58.9 ± 4.2	112.4 ± 6.8	148.3 ± 7.9
	Dry Biomass (mg/seedling)	4.2 ± 0.4	9.9 ± 0.8	18.9 ± 1.4	25.1 ± 1.8
	Plant Height (cm)	3.8 ± 0.4	9.6 ± 0.8	16.8 ± 1.3	22.4 ± 1.7
8.2	Cotyledonary Leaf Opening (%)	12.7 ± 1.9	62.4 ± 4.1	82.5 ± 4.3	84.6 ± 4.0
	Fresh Biomass (mg/seedling)	21.3 ± 2.0	49.7 ± 3.8	94.2 ± 5.9	118.5 ± 6.8
	Dry Biomass (mg/seedling)	3.6 ± 0.4	8.3 ± 0.7	15.8 ± 1.2	19.9 ± 1.5
	Plant Height (cm)	3.1 ± 0.3	7.9 ± 0.7	13.9 ± 1.1	17.8 ± 1.4
9.0	Cotyledonary Leaf Opening (%)	0.0	28.6 ± 3.1	52.4 ± 4.0	58.7 ± 4.3
	Fresh Biomass (mg/seedling)	14.8 ± 1.5	34.2 ± 2.9	62.8 ± 4.5	79.3 ± 5.3

	Dry Biomass (mg/seedling)	2.5 ± 0.3	5.7 ± 0.6	10.5 ± 0.9	13.3 ± 1.1
	Plant Height (cm)	1.8 ± 0.2	4.9 ± 0.5	8.9 ± 0.8	11.6 ± 1.0
10.2	Cotyledonary Leaf Opening (%)	0.0	12.3 ± 2.4	35.6 ± 3.5	41.2 ± 3.9
	Fresh Biomass (mg/seedling)	11.2 ± 1.2	26.4 ± 2.4	48.7 ± 3.9	62.4 ± 4.7
	Dry Biomass (mg/seedling)	1.9 ± 0.2	4.4 ± 0.5	8.2 ± 0.8	10.5 ± 0.9
	Plant Height (cm)	1.1 ± 0.2	3.2 ± 0.4	6.3 ± 0.6	8.7 ± 0.8
*Values are presented as Mean ± Standard Deviation (SD). All experiments were conducted in triplicate with 10 seedlings per replicate. Cotyledonary leaf opening is expressed as percentage of seedlings with fully opened cotyledonary leaves. Hyphen (-) indicates no germination/seedling survival. Optimal values at pH 7.0 are highlighted in bold. *					

At pH 5.2, seedling development was severely compromised. Cotyledonary leaf opening was completely absent until day 10, reaching only 10.5% by day 10 and ultimately 42.3% by day 20. Fresh biomass accumulation progressed slowly from 12.4 mg/seedling at day 5 to 58.9 mg/seedling at day 20, with corresponding dry biomass of 2.1 mg to 9.8 mg. Plant height reached only 9.2 cm by day 20, representing approximately 41% of the height achieved at optimal pH. This substantial growth suppression under mild acidity reflects the combined effects of aluminum toxicity and nutrient limitations documented by Kretzschmar et al. (1991).

At pH 6.2, substantial improvement in all parameters was observed. Cotyledonary leaf opening reached 8.3% by day 5 and progressed to 74.1% by day 20. Fresh biomass accumulation increased nearly eight-fold from 18.9 mg to 94.7 mg over the observation period, while dry biomass increased from 3.2 mg to 16.1 mg. Plant height reached 14.8 cm by day 20, approximately 66% of optimal height. This marked improvement demonstrates that even small pH adjustments toward neutrality substantially enhance seedling development.

Optimal seedling development was unequivocally observed at pH 7.0. Cotyledonary leaf opening reached 18.5% by day 5 and progressed rapidly to 78.3% by day 10 and 98.2% by day 20, indicating nearly complete transition to photosynthetic competence. Fresh biomass accumulation was exceptional, increasing from 24.6 mg/seedling at day 5 to 148.3 mg/seedling at day 20: a six-fold increase over the 15-day period. Dry biomass followed a similar trajectory, reaching 25.1 mg/seedling by day 20. Plant height attained 22.4 cm by day 20, representing vigorous seedling development. This optimal performance at neutral pH aligns with the findings of Davis et al. (1993) and Ahire et al. (2015), confirming that near-neutral conditions maximize metabolic efficiency and resource allocation to growth.

At pH 8.2, growth parameters remained substantial but reduced relative to optimal conditions. Cotyledonary leaf opening reached 84.6% by day 20, fresh biomass peaked at 118.5 mg/seedling (80% of optimal), dry biomass at 19.9 mg/seedling (79% of optimal), and plant height at 17.8 cm (79% of optimal). This mild alkaline stress primarily affected biomass accumulation rather than developmental progression, suggesting that metabolic efficiency rather than developmental programming is impaired under moderate alkalinity.

At pH 9.0, substantial growth suppression was evident. Cotyledonary leaf opening was completely absent until day 10, reaching only 28.6% by day 10 and 58.7% by day 20. Fresh biomass reached 79.3 mg/seedling (53% of optimal), dry biomass 13.3 mg/seedling (53% of optimal), and plant height 11.6 cm (52% of optimal). The most severe alkaline stress (pH 10.2) reduced all parameters further, with cotyledonary leaf opening reaching only 41.2%, fresh biomass 62.4 mg/seedling (42% of optimal), dry biomass 10.5 mg/seedling (42% of optimal), and plant height 8.7 cm (39% of optimal). The progressive decline with increasing alkalinity reflects impaired nutrient availability and potential direct pH effects on cellular metabolism (Sharma and Gupta, 2017).

Pusa 383 Seedling Development

Table 6 presents corresponding data for Pusa 383 across the same pH range. As with Pusa 334, complete failure occurred at pH 4.2 with no germination or survival.

Table 6: Effect of different pH levels on cotyledonary leaf opening, seedling biomass, and plant height of pearl millet (*Pennisetum glaucum*) variety Pusa 383 (20 days observation)

pH Level	Parameter	Day 5	Day 10	Day 15	Day 20
4.2	Cotyledonary Leaf Opening (%)	0.0	0.0	0.0	0.0
	Fresh Biomass (mg/seedling)	-	-	-	-
	Dry Biomass (mg/seedling)	-	-	-	-
	Plant Height (cm)	-	-	-	-
5.2	Cotyledonary Leaf Opening (%)	0.0	8.7 ± 1.9	32.4 ± 3.2	38.5 ± 3.6
	Fresh Biomass (mg/seedling)	10.8 ± 1.1	24.3 ± 2.2	40.6 ± 3.4	52.7 ± 4.1
	Dry Biomass (mg/seedling)	1.8 ± 0.2	4.1 ± 0.4	6.8 ± 0.6	8.8 ± 0.8
	Plant Height (cm)	1.0 ± 0.1	3.0 ± 0.3	5.9 ± 0.5	8.1 ± 0.7
6.2	Cotyledonary Leaf Opening (%)	6.9 ± 1.3	40.2 ± 3.7	68.5 ± 4.5	71.3 ± 4.4
	Fresh Biomass (mg/seedling)	16.5 ± 1.6	38.6 ± 3.2	71.2 ± 4.9	86.5 ± 5.7
	Dry Biomass (mg/seedling)	2.8 ± 0.3	6.5 ± 0.6	11.9 ± 1.0	14.5 ± 1.2
	Plant Height (cm)	2.1 ± 0.2	5.9 ± 0.5	10.2 ± 0.9	13.3 ± 1.1
7.0	Cotyledonary Leaf Opening (%)	**15.8 ± 2.0**	**72.6 ± 4.3**	**93.5 ± 2.8**	**96.4 ± 2.0**
	Fresh Biomass (mg/seedling)	**21.4 ± 1.9**	**52.3 ± 3.9**	**101.8 ± 6.2**	**134.6 ± 7.2**
	Dry Biomass (mg/seedling)	**3.6 ± 0.3**	**8.8 ± 0.7**	**17.1 ± 1.3**	**22.7 ± 1.6**
	Plant Height (cm)	**3.3 ± 0.3**	**8.5 ± 0.7**	**15.1 ± 1.2**	**20.2 ± 1.5**
8.2	Cotyledonary Leaf Opening (%)	10.4 ± 1.7	54.8 ± 3.9	75.6 ± 4.2	78.9 ± 4.1
	Fresh Biomass (mg/seedling)	18.7 ± 1.7	43.5 ± 3.4	84.7 ± 5.3	106.8 ± 6.3
	Dry Biomass (mg/seedling)	3.1 ± 0.3	7.3 ± 0.6	14.2 ± 1.1	17.9 ± 1.3
	Plant Height (cm)	2.7 ± 0.3	6.9 ± 0.6	12.3 ± 1.0	15.9 ± 1.3
9.0	Cotyledonary Leaf Opening (%)	0.0	22.4 ± 2.8	46.8 ± 3.8	53.2 ± 4.1
	Fresh Biomass (mg/seedling)	12.6 ± 1.3	29.5 ± 2.6	55.3 ± 4.1	70.8 ± 4.9
	Dry Biomass (mg/seedling)	2.1 ± 0.2	4.9 ± 0.5	9.3 ± 0.8	11.9 ± 1.0
	Plant Height (cm)	1.5 ± 0.2	4.1 ± 0.4	7.6 ± 0.7	10.1 ± 0.9
10.2	Cotyledonary Leaf Opening (%)	0.0	9.6 ± 2.0	30.2 ± 3.1	36.8 ± 3.5
	Fresh Biomass (mg/seedling)	9.7 ± 1.0	22.8 ± 2.1	42.5 ± 3.6	55.3 ± 4.3
	Dry Biomass (mg/seedling)	1.6 ± 0.2	3.8 ± 0.4	7.1 ± 0.7	9.3 ± 0.8
	Plant Height (cm)	0.9 ± 0.1	2.7 ± 0.3	5.4 ± 0.5	7.6 ± 0.7

*Values are presented as Mean ± Standard Deviation (SD). All experiments were conducted in triplicate with 10 seedlings per replicate. Cotyledonary leaf opening is expressed as percentage of seedlings with fully opened cotyledonary leaves. Hyphen (-) indicates no germination/seedling survival. Optimal values at pH 7.0 are highlighted in bold. *

At pH 5.2, Pusa 383 exhibited slightly lower performance than Pusa 334 across all parameters. Cotyledonary leaf opening reached only 38.5% by day 20 (compared to 42.3% for Pusa 334). Fresh biomass attained 52.7 mg/seedling (89% of Pusa 334's value at same pH), dry biomass 8.8 mg/seedling (90% of Pusa 334), and plant height 8.1 cm (88% of Pusa 334). This consistent differential suggests that the larger seed size of Pusa 334 provides greater metabolic reserves to sustain growth under acidic stress, supporting the seed size advantage hypothesis (Lafond and Baker, 1986).

At pH 6.2, the performance differential persisted though narrowed slightly. Pusa 383 achieved 71.3% cotyledonary leaf opening (96% of Pusa 334's 74.1%), fresh biomass 86.5 mg/seedling (91% of Pusa 334), dry biomass 14.5 mg/seedling (90% of Pusa 334), and plant height 13.3 cm (90% of Pusa 334). The narrowing differential suggests that as conditions approach optimal, the seed size advantage becomes less critical for sustaining growth.

At optimal pH 7.0, Pusa 383 achieved excellent seedling development, though still slightly below Pusa 334. Cotyledonary leaf opening reached 96.4% (98% of Pusa 334's 98.2%), fresh biomass 134.6 mg/seedling (91% of Pusa 334), dry biomass 22.7 mg/seedling (90% of Pusa 334), and plant

height 20.2 cm (90% of Pusa 334). The consistent ~10% reduction in biomass parameters for Pusa 383 relative to Pusa 334 across optimal conditions directly reflects the seed size difference, with Pusa 334's 22% greater seed mass (1.40 g vs 1.15 g per 100 seeds) translating into proportionally greater seedling biomass.

At pH 8.2, Pusa 383 maintained the same relative performance pattern, achieving 78.9% cotyledonary leaf opening (93% of Pusa 334's 84.6%), fresh biomass 106.8 mg/seedling (90% of Pusa 334), dry biomass 17.9 mg/seedling (90% of Pusa 334), and plant height 15.9 cm (89% of Pusa 334). At pH 9.0 and 10.2, the proportional relationships remained consistent, with Pusa 383 consistently achieving approximately 90% of Pusa 334's biomass accumulation and height.

The consistent pattern of Pusa 334 outperforming Pusa 383 across all pH conditions suggests that genetic differences, particularly those related to seed size and associated metabolic reserves, fundamentally determine stress tolerance and growth potential. This interpretation aligns with Duarte et al. (2015), who identified specific protein expression patterns associated with stress tolerance in millet seeds, and with Bailly et al. (2002), who demonstrated the importance of enzymatic antioxidant systems in mediating stress responses during germination and early establishment.

Effect of Temperature on Seedling Growth Parameters

Pusa 334 Seedling Development

Table 7 presents comprehensive temperature response data for Pusa 334 across eight temperature regimes. At 5°C, seedling development was severely retarded, with cotyledonary leaf opening completely absent until day 15, reaching only 8.4% by day 15 and 28.6% by day 20. Fresh biomass accumulation progressed extremely slowly from 4.2 mg/seedling at day 5 to merely 29.7 mg/seedling at day 20—representing only 19% of the biomass achieved at optimal temperature. Dry biomass followed a similar pattern, reaching 5.0 mg/seedling by day 20 (19% of optimal). Plant height attained only 4.6 cm (19% of optimal). This profound suppression at 5°C reflects near-complete inhibition of metabolic processes, consistent with the findings of Garcia-Huidobro et al. (1982) on temperature thresholds for millet germination and growth.

Table 7: Effect of different temperature regimes on cotyledonary leaf opening, seedling biomass, and plant height of pearl millet (*Pennisetum glaucum*) variety Pusa 334 (20 days observation):

Temp. (°C)	Parameter	Day 5	Day 10	Day 15	Day 20
5	Cotyledonary Leaf Opening (%)	0.0	0.0	8.4 ± 1.6	28.6 ± 3.0
	Fresh Biomass (mg/seedling)	4.2 ± 0.5	8.9 ± 0.9	16.3 ± 1.5	29.7 ± 2.6
	Dry Biomass (mg/seedling)	0.7 ± 0.1	1.5 ± 0.2	2.7 ± 0.3	5.0 ± 0.5
	Plant Height (cm)	0.4 ± 0.1	1.1 ± 0.2	2.4 ± 0.3	4.6 ± 0.5
10	Cotyledonary Leaf Opening (%)	0.0	12.5 ± 2.2	45.3 ± 3.8	72.8 ± 4.5
	Fresh Biomass (mg/seedling)	8.7 ± 0.9	21.4 ± 2.0	43.6 ± 3.5	74.2 ± 5.0
	Dry Biomass (mg/seedling)	1.5 ± 0.2	3.6 ± 0.4	7.3 ± 0.7	12.5 ± 1.1
	Plant Height (cm)	0.9 ± 0.1	2.8 ± 0.3	5.9 ± 0.6	9.8 ± 0.9
15	Cotyledonary Leaf Opening (%)	5.2 ± 1.1	38.6 ± 3.5	76.4 ± 4.5	88.2 ± 4.1
	Fresh Biomass (mg/seedling)	14.3 ± 1.4	38.5 ± 3.2	79.8 ± 5.3	118.6 ± 6.7
	Dry Biomass (mg/seedling)	2.4 ± 0.3	6.5 ± 0.6	13.4 ± 1.1	19.9 ± 1.5
	Plant Height (cm)	1.8 ± 0.2	5.2 ± 0.5	10.6 ± 0.9	15.8 ± 1.3
20	Cotyledonary Leaf Opening (%)	12.8 ± 1.9	68.5 ± 4.2	94.2 ± 2.5	98.5 ± 1.2
	Fresh Biomass (mg/seedling)	21.5 ± 2.0	54.7 ± 4.0	108.3 ± 6.4	156.8 ± 8.1

	Dry Biomass (mg/seedling)	3.6 ± 0.4	9.2 ± 0.8	18.2 ± 1.4	26.3 ± 1.9
	Plant Height (cm)	3.2 ± 0.3	8.7 ± 0.8	16.2 ± 1.3	23.5 ± 1.8
25	Cotyledonary Leaf Opening (%)	15.6 ± 2.1	72.4 ± 4.3	93.8 ± 2.8	97.2 ± 1.8
	Fresh Biomass (mg/seedling)	23.8 ± 2.2	58.2 ± 4.2	112.5 ± 6.7	159.4 ± 8.3
	Dry Biomass (mg/seedling)	4.0 ± 0.4	9.8 ± 0.8	18.9 ± 1.5	26.8 ± 2.0
	Plant Height (cm)	3.5 ± 0.4	9.2 ± 0.8	16.8 ± 1.4	24.1 ± 1.9
30	Cotyledonary Leaf Opening (%)	10.4 ± 1.7	58.7 ± 4.0	85.6 ± 4.2	89.3 ± 3.9
	Fresh Biomass (mg/seedling)	19.6 ± 1.8	48.3 ± 3.7	94.7 ± 5.8	132.5 ± 7.1
	Dry Biomass (mg/seedling)	3.3 ± 0.3	8.1 ± 0.7	15.9 ± 1.2	22.2 ± 1.6
	Plant Height (cm)	2.8 ± 0.3	7.5 ± 0.7	14.1 ± 1.1	19.8 ± 1.5
35	Cotyledonary Leaf Opening (%)	0.0	32.4 ± 3.2	58.7 ± 4.1	64.5 ± 4.3
	Fresh Biomass (mg/seedling)	11.5 ± 1.2	28.6 ± 2.5	56.3 ± 4.2	78.9 ± 5.2
	Dry Biomass (mg/seedling)	1.9 ± 0.2	4.8 ± 0.5	9.4 ± 0.8	13.2 ± 1.1
	Plant Height (cm)	1.4 ± 0.2	3.9 ± 0.4	7.8 ± 0.7	10.9 ± 0.9
40	Cotyledonary Leaf Opening (%)	0.0	0.0	6.2 ± 1.3	12.8 ± 2.2
	Fresh Biomass (mg/seedling)	3.8 ± 0.4	7.2 ± 0.8	12.5 ± 1.2	18.6 ± 1.7
	Dry Biomass (mg/seedling)	0.6 ± 0.1	1.2 ± 0.2	2.1 ± 0.3	3.1 ± 0.4
	Plant Height (cm)	0.3 ± 0.1	0.9 ± 0.1	1.8 ± 0.2	2.7 ± 0.3

*Values are presented as Mean ± Standard Deviation (SD). All experiments were conducted in triplicate with 10 seedlings per replicate. Cotyledonary leaf opening is expressed as percentage of seedlings with fully opened cotyledonary leaves. Optimal temperature range (20-25°C) is highlighted in bold. *

At 10°C, substantial improvement was evident. Cotyledonary leaf opening reached 12.5% by day 10 and progressed to 72.8% by day 20. Fresh biomass increased from 8.7 mg/seedling at day 5 to 74.2 mg/seedling at day 20 (47% of optimal), dry biomass reached 12.5 mg/seedling (47% of optimal), and plant height attained 9.8 cm (41% of optimal). This approximately 2.5-fold improvement over 5°C conditions demonstrates the steep temperature response curve in the low-temperature range.

At 15°C, seedling development approached optimal levels. Cotyledonary leaf opening reached 88.2% by day 20, fresh biomass attained 118.6 mg/seedling (74% of optimal), dry biomass 19.9 mg/seedling (74% of optimal), and plant height 15.8 cm (66% of optimal). The narrowing gap between parameters at 15°C and optimal temperatures indicates that the critical temperature threshold for near-normal development lies between 15-20°C for this variety.

The optimal temperature range was clearly 20-25°C, with both temperatures yielding statistically similar results across all parameters. At 20°C, cotyledonary leaf opening reached 98.5%, fresh biomass 156.8 mg/seedling, dry biomass 26.3 mg/seedling, and plant height 23.5 cm. At 25°C, corresponding values were 97.2%, 159.4 mg/seedling, 26.8 mg/seedling, and 24.1 cm. The slight shift toward marginally higher biomass at 25°C (approximately 2% higher) may reflect more rapid metabolic rates at the higher temperature, consistent with the temperature-rate relationships described by Garcia-Huidobro et al. (1982).

At 30°C, growth parameters declined noticeably. Cotyledonary leaf opening reached 89.3% (91% of optimal), fresh biomass 132.5 mg/seedling (83% of optimal), dry biomass 22.2 mg/seedling (83% of optimal), and plant height 19.8 cm (82% of optimal). This decline at 30°C contrasts with some literature reports suggesting optimal temperatures for pearl millet up to 34°C (Mohamed et al., 1988), indicating possible varietal differences in thermal optima.

At 35°C, substantial growth suppression occurred, with cotyledonary leaf opening reaching only 64.5% (66% of optimal), fresh biomass 78.9 mg/seedling (49% of optimal), dry biomass 13.2 mg/seedling (49% of optimal), and plant height 10.9 cm (45% of optimal). The most severe suppression was observed at 40°C, where all parameters were reduced to minimal levels: cotyledonary leaf opening 12.8%, fresh biomass 18.6 mg/seedling (12% of optimal), dry biomass 3.1 mg/seedling (12% of optimal), and plant height 2.7 cm (11% of optimal). This dramatic decline at 40°C reflects protein denaturation and enzyme inactivation at supraoptimal temperatures (Duarte et al., 2015).

Pusa 383 Seedling Development

Table 8 presents temperature response data for Pusa 383, revealing patterns generally similar to Pusa 334 but with consistently lower absolute values.

Table 8: Effect of different temperature regimes on cotyledonary leaf opening, seedling biomass, and plant height of pearl millet (*Pennisetum glaucum*) variety Pusa 383 (20 days observation):

Temp. (°C)	Parameter	Day 5	Day 10	Day 15	Day 20
5	Cotyledonary Leaf Opening (%)	0.0	0.0	6.2 ± 1.3	23.4 ± 2.8
	Fresh Biomass (mg/seedling)	3.6 ± 0.4	7.5 ± 0.8	13.8 ± 1.3	25.2 ± 2.3
	Dry Biomass (mg/seedling)	0.6 ± 0.1	1.3 ± 0.2	2.3 ± 0.3	4.2 ± 0.4
	Plant Height (cm)	0.3 ± 0.1	0.9 ± 0.1	2.0 ± 0.2	3.9 ± 0.4
10	Cotyledonary Leaf Opening (%)	0.0	9.8 ± 1.9	38.6 ± 3.5	64.5 ± 4.2
	Fresh Biomass (mg/seedling)	7.2 ± 0.8	18.3 ± 1.7	37.8 ± 3.2	65.4 ± 4.6
	Dry Biomass (mg/seedling)	1.2 ± 0.2	3.1 ± 0.3	6.3 ± 0.6	11.0 ± 1.0
	Plant Height (cm)	0.7 ± 0.1	2.3 ± 0.2	5.0 ± 0.5	8.5 ± 0.8
15	Cotyledonary Leaf Opening (%)	4.1 ± 0.9	32.5 ± 3.2	68.9 ± 4.3	82.6 ± 4.0
	Fresh Biomass (mg/seedling)	12.4 ± 1.2	33.8 ± 3.0	70.5 ± 4.8	105.8 ± 6.2
	Dry Biomass (mg/seedling)	2.1 ± 0.2	5.7 ± 0.6	11.8 ± 1.0	17.7 ± 1.4
	Plant Height (cm)	1.5 ± 0.2	4.5 ± 0.4	9.3 ± 0.8	14.0 ± 1.2
20	Cotyledonary Leaf Opening (%)	10.5 ± 1.7	61.2 ± 4.0	91.5 ± 3.0	96.8 ± 1.6
	Fresh Biomass (mg/seedling)	18.6 ± 1.7	48.2 ± 3.6	97.6 ± 5.8	142.3 ± 7.5
	Dry Biomass (mg/seedling)	3.1 ± 0.3	8.1 ± 0.7	16.4 ± 1.3	23.9 ± 1.7
	Plant Height (cm)	2.8 ± 0.3	7.6 ± 0.7	14.5 ± 1.2	21.2 ± 1.6
25	Cotyledonary Leaf Opening (%)	13.2 ± 1.9	65.8 ± 4.1	90.8 ± 3.2	95.5 ± 2.1
	Fresh Biomass (mg/seedling)	20.5 ± 1.9	51.6 ± 3.8	101.4 ± 6.1	144.8 ± 7.7
	Dry Biomass (mg/seedling)	3.4 ± 0.3	8.7 ± 0.7	17.0 ± 1.3	24.3 ± 1.8
	Plant Height (cm)	3.0 ± 0.3	8.1 ± 0.7	15.2 ± 1.2	21.9 ± 1.7
30	Cotyledonary Leaf Opening (%)	8.5 ± 1.5	49.6 ± 3.8	78.4 ± 4.3	83.7 ± 4.0
	Fresh Biomass (mg/seedling)	16.8 ± 1.6	42.5 ± 3.4	84.6 ± 5.3	118.9 ± 6.5
	Dry Biomass (mg/seedling)	2.8 ± 0.3	7.1 ± 0.6	14.2 ± 1.1	19.9 ± 1.5
	Plant Height (cm)	2.4 ± 0.2	6.5 ± 0.6	12.5 ± 1.0	17.6 ± 1.4
35	Cotyledonary Leaf Opening (%)	0.0	26.8 ± 2.9	51.2 ± 3.9	58.3 ± 4.1
	Fresh Biomass (mg/seedling)	9.6 ± 1.0	24.3 ± 2.2	48.7 ± 3.8	68.5 ± 4.7
	Dry Biomass (mg/seedling)	1.6 ± 0.2	4.1 ± 0.4	8.2 ± 0.7	11.5 ± 1.0
	Plant Height (cm)	1.2 ± 0.1	3.3 ± 0.3	6.7 ± 0.6	9.5 ± 0.8
40	Cotyledonary Leaf Opening (%)	0.0	0.0	4.8 ± 1.1	9.6 ± 1.8
	Fresh Biomass (mg/seedling)	3.2 ± 0.3	6.1 ± 0.6	10.4 ± 1.0	15.8 ± 1.5
	Dry Biomass (mg/seedling)	0.5 ± 0.1	1.0 ± 0.1	1.7 ± 0.2	2.6 ± 0.3
	Plant Height (cm)	0.2 ± 0.1	0.7 ± 0.1	1.5 ± 0.2	2.3 ± 0.2

*Values are presented as Mean ± Standard Deviation (SD). All experiments were conducted in triplicate with 10 seedlings per replicate. Cotyledonary leaf opening is expressed as percentage of

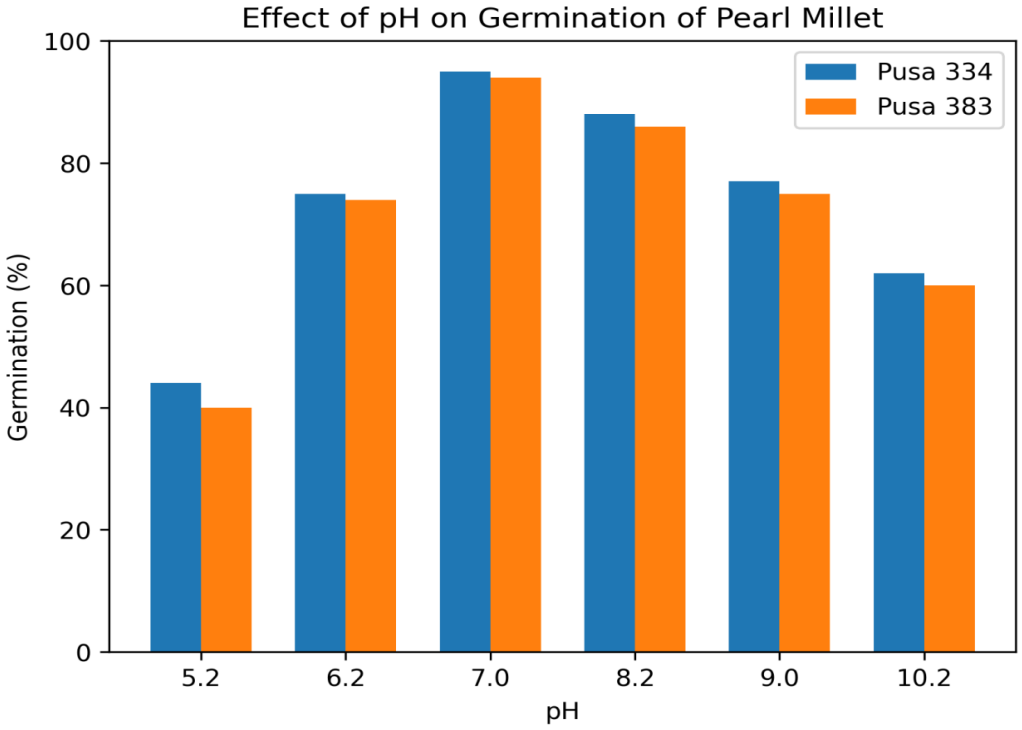
seedlings with fully opened cotyledonary leaves. Optimal temperature range (20-25°C) is highlighted in bold. *

At 5°C, Pusa 383 reached 23.4% cotyledonary leaf opening by day 20 (82% of Pusa 334's 28.6%), fresh biomass 25.2 mg/seedling (85% of Pusa 334), dry biomass 4.2 mg/seedling (84% of Pusa 334), and plant height 3.9 cm (85% of Pusa 334). This pattern of Pusa 383 achieving approximately 82-85% of Pusa 334's values persisted across all temperature treatments.

At 10°C, Pusa 383 reached 64.5% cotyledonary leaf opening (89% of Pusa 334's 72.8%), fresh biomass 65.4 mg/seedling (88% of Pusa 334), dry biomass 11.0 mg/seedling (88% of Pusa 334), and plant height 8.5 cm (87% of Pusa 334). At 15°C, corresponding values were 82.6% (94% of Pusa 334's 88.2%), 105.8 mg/seedling (89% of Pusa 334), 17.7 mg/seedling (89% of Pusa 334), and 14.0 cm (89% of Pusa 334).

At optimal temperatures (20-25°C), the proportional relationships remained consistent. At 20°C, Pusa 383 achieved 96.8% cotyledonary leaf opening (98% of Pusa 334's 98.5%), fresh biomass 142.3 mg/seedling (91% of Pusa 334), dry biomass 23.9 mg/seedling (91% of Pusa 334), and plant height 21.2 cm (90% of Pusa 334). At 25°C, corresponding values were 95.5% (98% of Pusa 334's 97.2%), 144.8 mg/seedling (91% of Pusa 334), 24.3 mg/seedling (91% of Pusa 334), and 21.9 cm (91% of Pusa 334).

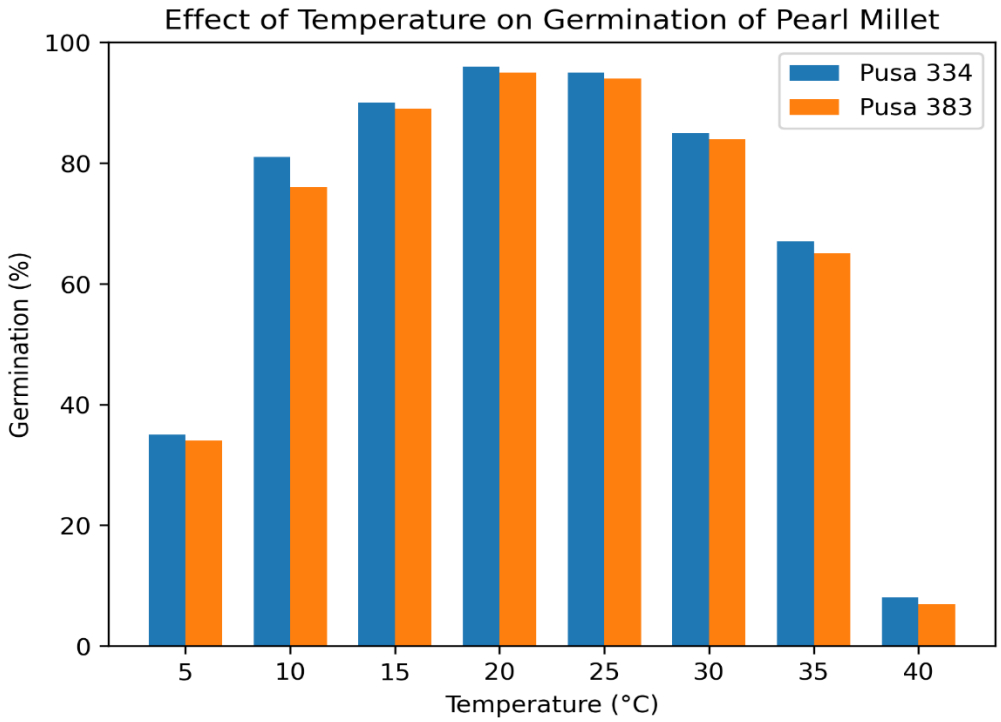
At supraoptimal temperatures (30-40°C), the pattern persisted with Pusa 383 maintaining approximately 90% of Pusa 334's biomass and height values. At 30°C, Pusa 383 achieved 83.7% cotyledonary leaf opening (94% of Pusa 334's 89.3%), fresh biomass 118.9 mg/seedling (90% of Pusa 334), dry biomass 19.9 mg/seedling (90% of Pusa 334), and plant height 17.6 cm (89% of Pusa 334). At 35°C, values were 58.3% (90% of Pusa 334's 64.5%), 68.5 mg/seedling (87% of Pusa 334), 11.5 mg/seedling (87% of Pusa 334), and 9.5 cm (87% of Pusa 334). At 40°C, the most severe stress conditions, Pusa 383 reached 9.6% cotyledonary leaf opening (75% of Pusa 334's 12.8%), fresh biomass 15.8 mg/seedling (85% of Pusa 334), dry biomass 2.6 mg/seedling (84% of Pusa 334), and plant height 2.3 cm (85% of Pusa 334).



Synthesis and Implications

The comprehensive dataset reveals several important patterns with implications for pearl millet cultivation and breeding. First, both varieties demonstrated optimal germination and seedling development at pH 7.0 and temperatures between 20-25°C, confirming the importance of near-

neutral soil conditions and moderate temperatures for maximizing establishment success (Ahire et al., 2015; Srivastava and Singh, 2004). The complete failure at pH 4.2 and near-complete failure at 40°C establish clear stress thresholds beyond which these varieties cannot establish successfully.



Second, Pusa 334 consistently outperformed Pusa 383 across all treatment conditions, with the performance differential most pronounced in biomass accumulation parameters. The consistent ~10% advantage in biomass for Pusa 334 correlates directly with its 22% greater seed mass (1.40 g vs 1.15 g per 100 seeds), supporting the hypothesis that larger seed reserves provide sustained metabolic advantage throughout early development (Lafond and Baker, 1986; Giraldo et al., 2008). This seed size advantage appears particularly beneficial under stress conditions, where the differential was most pronounced.

Third, the temporal patterns of cotyledonary leaf opening provide insight into developmental progression under stress. At optimal conditions, both varieties achieved >95% leaf opening by day 15-20, indicating successful transition to photosynthetic competence. Under suboptimal pH or temperature, this progression was delayed and reduced, with the magnitude of delay corresponding to stress intensity. This pattern reflects the integrated effects of environmental conditions on metabolic processes governing seedling development (Soltani et al., 2006).

Fourth, the reproductive capacity calculations (Table 2) provide a useful integrative measure of varietal performance potential. Pusa 334's higher reproductive capacity (1907.22 vs 1736.46) reflects both greater seed production (1850 vs 1667 seeds/plant) and marginally higher germination percentage (97% vs 96%). This reproductive advantage, combined with superior seedling vigor under stress, suggests that Pusa 334 may be better suited for environments where suboptimal conditions during establishment are likely.

The physiological mechanisms underlying these differential responses likely involve the enzymatic antioxidant systems documented by Bailly et al. (2002) and the stress-responsive proteins identified by Duarte et al. (2015). Larger seed size in Pusa 334 may provide greater initial investment in these protective systems, enabling more robust responses to pH and temperature stress during the critical establishment phase. Further investigation of these mechanisms at the biochemical and molecular levels would provide valuable insights for breeding programs targeting enhanced stress tolerance.

Conclusion

Pusa 334 outperformed Pusa 383 across all pH and temperature regimes, with optimal germination and seedling development at pH 7.0 and 20-25°C. The 22% larger seed mass of Pusa 334 conferred consistent advantages in biomass accumulation and stress tolerance, establishing it as the superior variety for cultivation under diverse environmental conditions.

Conflict of Interest

The authors declare that they have no conflict of interest.

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