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Original Article

POLLEN VIABILITY AND GERMINATION RESPONSES OF CASHEW (*ANACARDIUM OCCIDENTALE* L.) UNDER IN VITRO CONDITIONS

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ABSTRACT

Anacardium occidentale L. (cashew) serves as an essential commercial crop in tropical and subtropical areas, valued for its economic significance and nutritional benefits. Pollen is crucial in plant reproduction, with viability and germination determining fertilization success. However, variations in pollen viability among cashew accessions remain understudied. Understanding genetic variability in pollen fertility will aid in selecting superior pollen donors, enhance cross-breeding efficiency, and supports the development of improved cashew cultivars for sustainable production. This study investigated pollen viability and optimized in vitro germination conditions for 18 cashew clones. The research aimed to determine the efficacy of pollen from different clones and establish optimal protocols for successful in vitro pollen germination. The findings are expected to contribute to improved cashew breeding strategies and enhanced genetic resource management. At the Cocoa Research Institute of Nigeria (CRIN), Ibadan, pollen viability was evaluated via aniline blue-lactophenol staining. In vitro pollen germination was assessed using modified Brewbaker and Kwack's (1964) media, with observations recorded after a 24-hour incubation. Pollen tube lengths were subsequently measured using ImageJ, and the resultant data were subjected to ANOVA analysis, followed by Duncan's Multiple Range Test for mean separation. Pollen viability analysis revealed significant inter-accession variation, from 56.3% to 97.2%. Accessions EU202 (97.2%) and CR102 (96.1%) demonstrated superior viability, while Brazilian Medium (56.3%) and India Madras (59.5%) exhibited comparatively poor performance. Germination rates also varied considerably, with select clones achieving complete germination in Media 2. Statistical analysis confirmed genotype-specific influences on pollen germination in response to differing media conditions.

Key words: Cashew, Pollen tube growth, cavity slide, invitro germination, aniline blue staining

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a vital cash crop in many tropical and subtropical regions, valued for its economic importance and nutritional benefits [1]. Despite the increasing global demand for cashew nuts, the industry faces several challenges, including limited availability of improved cultivars with desirable traits such as high yield, disease resistance, and early maturity. Effective breeding programs are essential to address these challenges and enhance cashew production [2] However, conventional hybridization techniques, though widely used, are time-consuming and constrained by the availability of suitable parental lines [3]. Advancements in reproductive biology, particularly studies on pollen viability and in vitro germination, offer new opportunities for accelerating cashew breeding efforts.

Pollen plays a critical role in plant reproduction, serving as the male gametophyte responsible for fertilization. Pollen viability, which refers to the ability of pollen grains to effect fertilization, is an essential determinant of successful hybridization and fruit production [4]. Studies have shown that pollen viability can vary among species and cultivars, influenced by factors such as ploidy level and hybridity [5]. In vitro pollen germination techniques are reliable for assessing pollen quality and determining germination potential, making them essential tools in breeding programs [6]. Unlike staining techniques, which may overestimate viability, in vitro germination tests directly measure the pollen tube development under controlled conditions, offering more accurate assessments of fertility [6].

Due to their conservative morphological features, including symmetry, shape, apertural patterns, and exine configuration, pollen have been extensively studied in angiosperm taxonomy and phylogeny [7]. These characteristics can provide insights into genetic diversity and evolutionary relationships among cashew cultivars. Moreover, effective pollination is a prerequisite for fruit and seed production, with pollen viability and tube growth being key determinants of reproductive success [8].

In cashew, the andromonoecious flowering system, where male (staminate) and hermaphroditic (perfect) flowers coexist on the same panicle, necessitates a thorough understanding of pollen biology to optimize breeding strategies [9]. Despite the significance of pollen viability and in vitro germination in cashew breeding, limited studies have explored these aspects in different genotypes, highlighting the need for further research.

This study aims to evaluate pollen viability and optimize in vitro germination conditions across different cashew accessions. Understanding the genetic variability in pollen fertility and germination rates will contribute to the selection of superior pollen donors, enhance cross-breeding efficiency, and ultimately support the development of improved cashew cultivars. The findings of this research will provide valuable insights for breeding programs, facilitating the genetic improvement and sustainability of the cashew industry.

MATERIALS AND METHODS

Study site

The experiment was undertaken at the Cocoa Research Institute of Nigeria (CRIN) in Ibadan, situated within the humid tropical rainforest zone of Nigeria at coordinates 07° 10' 0'' N and 03° 52' 0'' E. The location is characterized by a yearly

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Table 1. List of Cashew Clones used in the study

S/N	Clones	S/N	Clones	S/N	Clones	S/N	Clones
1	OCJ2	6	OCJ9	11	EU202	16	BEL 22
2	KD202	7	KD102	12	Brazilian Medium	17	YS103
3	EN103	8	CR102	13	YO103	18	ORO9
4	India Madras	9	BEL36	14	India Large		
5	ORO3	10	YS203	15	India Medium		

precipitation range of 1,200 mm to 1,500 mm and a mean daily temperature of 30.1°C.

Plant Materials

Eighteen cashew (*Anacardium occidentale* L.) Clones were evaluated for pollen viability and in vitro germination, as presented in Table 1

Pollen Collection Protocols

Pollen grains were collected from healthy, mature flowers that were randomly selected from each of the 18 cashew clones listed in Table 1. To ensure consistency in pollen quality, only flowers that had fully opened in the morning (between 8:00 AM and 10:00 AM) were used. Fresh pollen was collected by gently tapping six anthers per flower into collection tubes containing a 1:3 solution of Glacial Acetic Acid (GAA) and 70% ethanol to preserve the pollen's viability for later analysis. The sample size for each clone was standardized to six flowers per clone to ensure uniformity in the experiment.

Environmental Conditions

Pollen collection and germination experiments were conducted under natural environmental conditions, with room temperature maintained at approximately 25°C and a relative humidity range of 60-70%. These conditions were consistent throughout the study to minimize environmental variability that could affect pollen viability and germination.

Pollen Viability and Germination

Pollen viability was assessed using the aniline blue-lactophenol staining method [10]. Pollen grains were freshly collected from six anthers per accession and transferred onto a glass slide. They were then gently pressed to facilitate their release. Aniline blue stain (1–2 drops) was applied and allowed to absorb for 30 minutes. A clean coverslip was carefully placed over the slide to minimize air bubbles. Viable pollen grains appeared dark blue, whereas nonviable ones remained either unstained or exhibited a light blue hue. Pollen viability was determined by counting 100 pollen grains per slide, and the viability percentage was computed using the following formula:

$$\text{Pollen viability (\%)} = \frac{\text{Number pollen grains that absorbed stain}}{\text{Total pollen grains examined}} \; X\,100$$

Pollen germination was assessed using two different media based on the Brewbaker and Kwack method [11]:

Medium 1: 10% sucrose, 100 mg/L boric acid, 100 mg/L potassium nitrate, 200 mg/L magnesium sulfate.

Medium 2: 10% sucrose, 100 mg/L boric acid, 150 mg/L potassium sulfate, 150 mg/L calcium chloride.

A cavity depression glass slide was used for pollen germination. The prepared media were pipetted into the cavity slide, and fresh cashew pollen grains were gently dropped onto the surface. The slides were incubated for 24 hours at room temperature under natural environmental conditions. A glass cover slip was placed over the slide before microscopic observation Pollen grains were classified as germinated when the pollen tube extended to at least twice the grain's diameter, following the criteria outlined by Wang *et al.* [12]. The percentage of pollen germination was evaluated by counting all observable pollen grains within a microscopic field and applying the following formula:

$$\text{Germination rate(\%)} = \frac{\text{Number of pollen grains that germinated}}{\text{Total pollen grains examined}} \; X\,100$$

Data Analysis

The viability and germination rates of the pollen were analyzed using analysis of variance (ANOVA) in the Statistical Analysis System (SAS) software, Version 9.1 (2003). Mean separation was conducted using Duncan's Multiple Range test at a significance level of 0.05. Pollen tube length was measured using ImageJ software, Version 1.54p (2025). Germination rate and pollen tube length were recorded 24 hours after exposure to the germination media

RESULTS

The viability assessment of 18 cashew clones, determined through the aniline blue staining method, as shown in Plate 1 revealed considerable variability among the clones. Pollen viability percentages as shown in Figure 1 ranged from 56.25% to 97.22%. The highest viability was recorded in EU202 (97.22%), followed by CR102 (96.05%) and EN103 (94.17%), indicating their strong reproductive potential. Other clones with high viability included BEL 22 (93.33%) and YS103 (93.75%), further supporting their potential suitability for breeding programs.

In contrast, some clones exhibited lower viability, including Brazilian Medium (56.25%), and India Madras (59.52%). The majority of the clones had viability above 80%, with notable examples such as KD202 (88.89%), India Medium (90.67%), and ORO9 (88.64%), demonstrating their potential for further propagation and genetic improvement programs.

The pollen germination percentages across two different media as shown in Figure 2 varied among the 18 cashew clones, revealing distinct responses to media composition. In Media 1, most clones exhibited moderate germination rates, with 50% germination observed in the majority of clones. Exceptions included BEL36 (0%), ORO3 (100%), YS103 (100%), ORO9 (25%), and BEL22 (65%).

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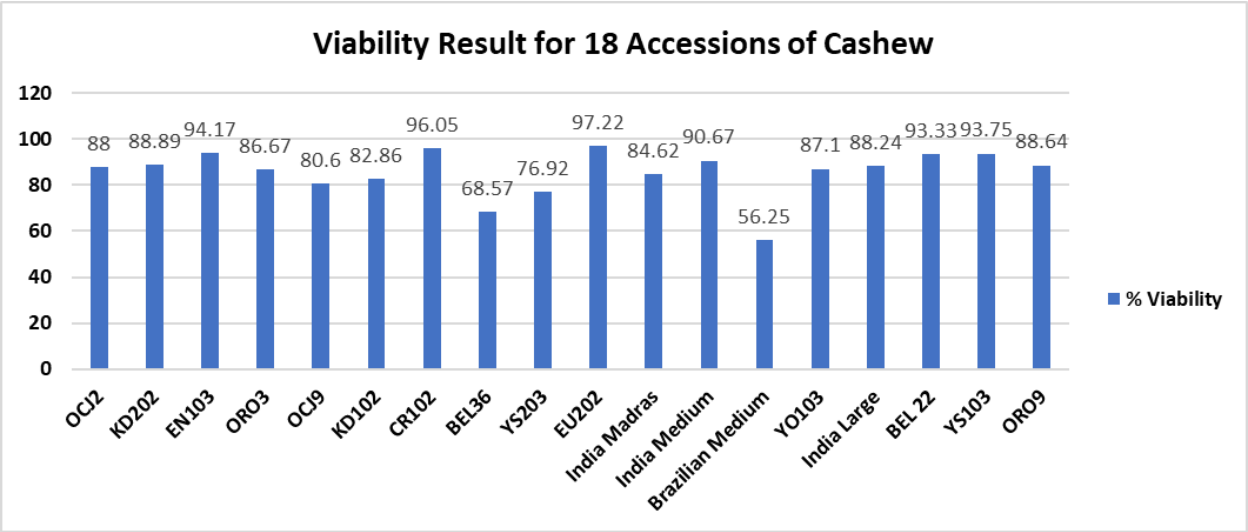

Figure 1. Pollen Viability (%) of 20 Cashew Accessions

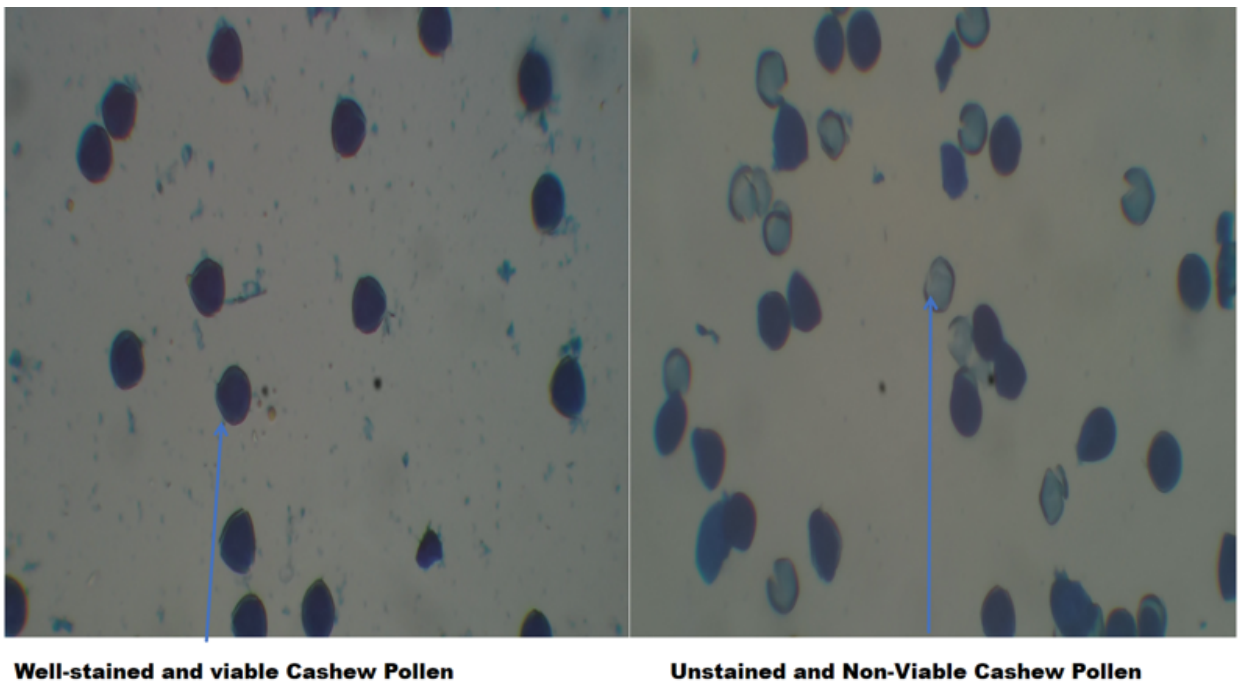

Plate 1. Visualization of Viable and Non-Viable Pollen in Cashew Clones Using Aniline Blue Staining

In Media 2, germination rates improved significantly for certain clones, with some achieving 100% germination, including India Madras, Brazilian Medium, India Medium, BEL36, YS103, and ORO9. However, some clones, such as ORO3 and YS203, exhibited zero germination in Media 2.

Clones with high viability percentages, such as EU202 (97.22%), CR102 (96.05%), and EN103 (94.17%), demonstrated consistent germination across both media. Similarly, YS103 and BEL22, with viability rates above 93%, exhibited robust germination, particularly in Media 2. Contrarily, clones with lower viability, such as Brazilian Medium (56.25%) and India Madras (59.52%), showed improved germination in Media 2, indicating a stronger response to more favorable conditions.

Most of the accessions presented in Table 2, along with the visualizations shown in Plates 2-5, exhibited normal pollen tube morphology in both media, indicating favorable conditions for pollen development. However, EU202 showed abnormal burst tubes (BT) in both media, while YO103 displayed branching (Br) pollen tubes in Media 2, suggesting structural deviations. BEL22 exhibited swollen tips (ST) in Media 1, but normal morphology in Media 2, indicating media influence on tube integrity. The pollen tube growth pattern varied across media, with most accessions demonstrating straight growth, which is indicative of normal elongation. However, OCJ2, KD102, and India Large showed curved tube growth in Media 1, while BEL36 and YO103 exhibited curvature in Media 2, suggesting differential media effects on directional tube elongation. Some accessions, such as ORO3 and YS203, showed no pollen tube growth in Media 2, while BEL36 exhibited no growth in Media 1, indicating unfavorable conditions for pollen tube development in these instances.

ST: Swollen tips BT: Burst tubes Br: Branching NG: No growth

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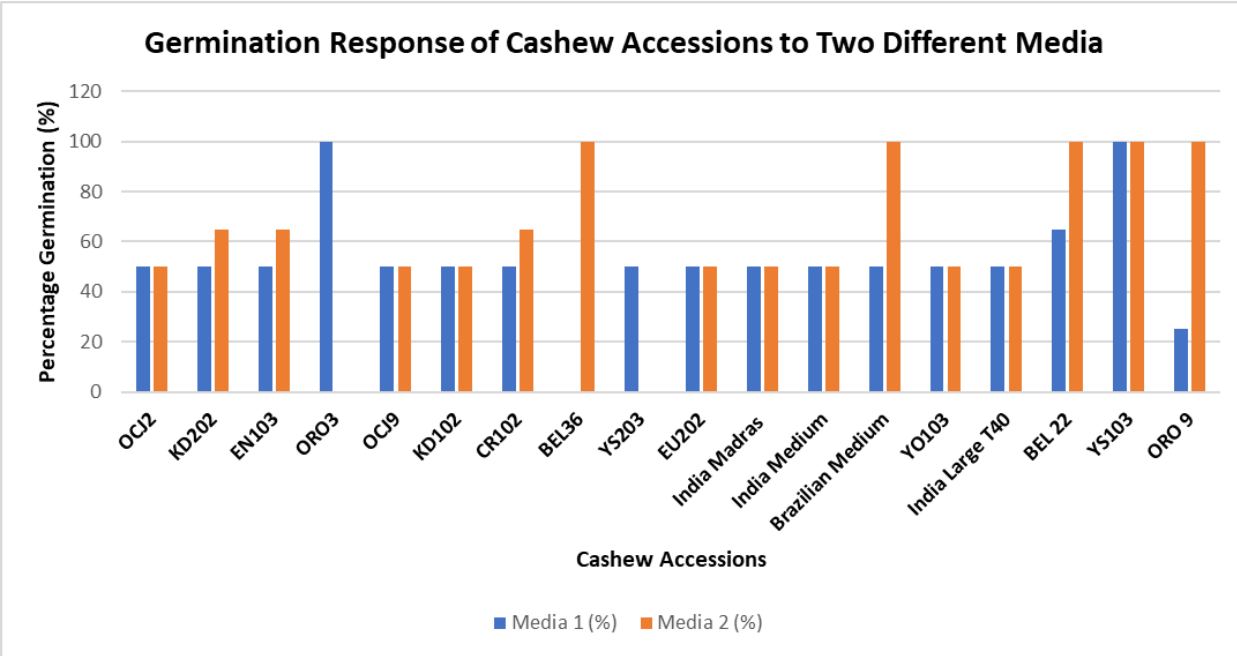

Figure 2. Comparative Germination Percentage of 20 Cashew Accessions in Two Different Media

Table 2. The qualitative evaluation of pollen tube morphology and growth behavior across different media revealed varying responses among the cashew accessions.

S/N	CLONES	Media 1	Media 2	Media 1	Media 2
		Tube Morphology (Normal or Abnormal		Growth Behaviour (Straight or Curve tubes)	
1	OCJ2	Normal	Normal	Curve	Straight
2	KD202	Normal	Normal	Straight	Curve
3	EN103	Normal	Normal	Straight	Straight
4	ORO3	Normal	NG	Straight	NG
5	OCJ9	Normal	Normal	Straight	Straight
6	KD102	Normal	Normal	Curve	Straight
7	CR102	Normal	Normal	Straight	Straight
8	BEL36	NG	Normal	NG	Curve
9	YS203	Normal	NG	Straight	NG
10	EU202	Abnormal (BT)	Abnormal (BT)	Straight	Straight
11	India Madras	Normal	Normal	Straight	Straight
12	India Medium	Normal	Normal	Straight	Straight
13	Brazilian Medium	Normal	Normal	Straight	Straight
14	YO103	Normal	Abnormal (Br)	Straight	Curve
15	India Large	Normal	Normal	Curve	Straight
16	BEL 22	Abnormal (ST)	Normal	Curve	Straight
17	YS103	Normal	Normal	Straight	Straight
18	ORO 9	Normal	Normal	Straight	Straight

The mean separation results based on Duncan's Multiple Range Test as shown in Table 3 reveal significant variations in pollen tube length ($p < 0.001$) and germination ($p < 0.01$) among the clones. At the same time, media composition remained statistically uniform across all clones (n.s).

The letters (A, B, C, D, E, F, G) represent the grouping of

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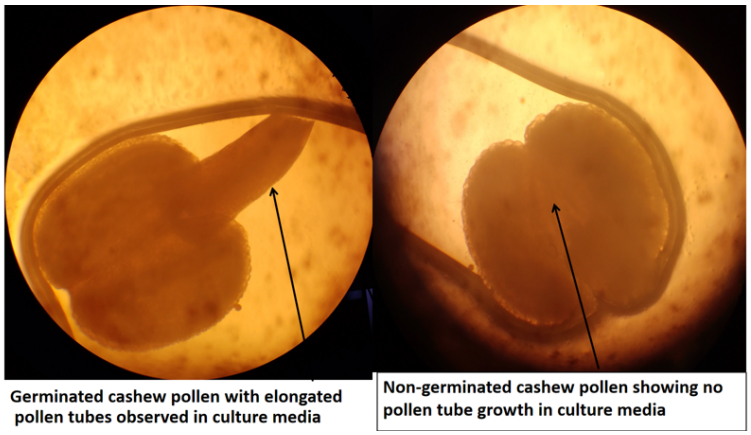

Plate 2. Visualization of germinated cashew pollen with elongated pollen tubes and non-germinated pollen

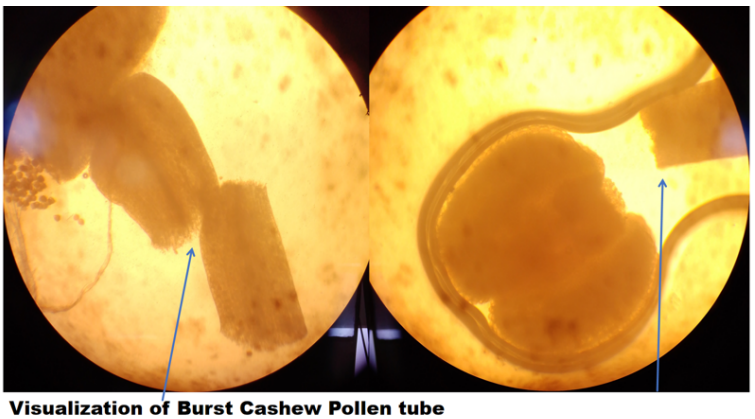

Plate 3. Visualization of burst Cashew pollen tubes

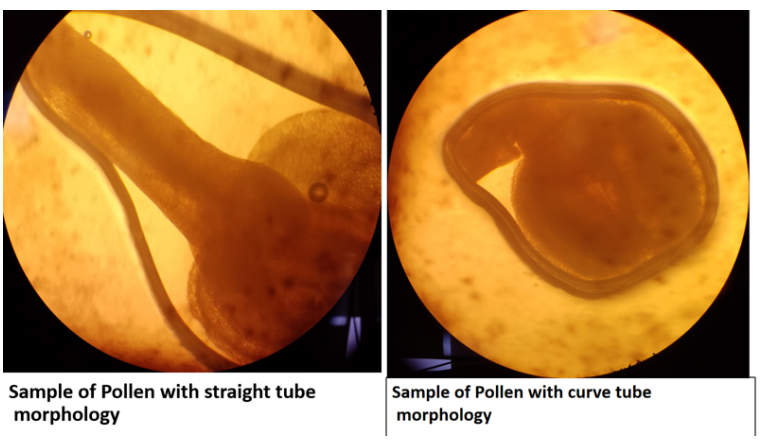

Plate 4. Visualization of sample of pollen with straight and curve tube

Plate 5. Visualization of sample of abnormal and normal pollen growth behaviour

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Table 3: Mean Separation Table for Media, Pollen Tube Length, and Germination Based on Duncan's Multiple Range Test

S/N	CLONES	Media	Pollen Tube Length (µm)	Germination (%)
1	BEL 22	1.5000 ^{A}	54.42 ^{FG}	79.83 ^{AB}
2	BEL36	1.5000 ^{A}	69.06 ^{FGDE}	49.50 ^{BC}
3	Brazilian Medium	1.5000 ^{A}	137.13 ^{B}	73.67 ^{AB}
4	CR102	1.5000 ^{A}	61.27 ^{FG}	57.33 ^{BC}
5	EN103	1.5000 ^{A}	77.40 ^{DEF}	56.33 ^{BC}
6	EU202	1.5000 ^{A}	177.89 ^{A}	51.67 ^{BC}
7	India Large	1.5000 ^{A}	61.58 ^{FG}	51.00 ^{BC}
8	India Madras	1.5000 ^{A}	103.52 ^{CD}	51.00 ^{BC}
9	India Medium	1.5000 ^{A}	70.32 ^{FGDE}	73.00 ^{AB}
10	KD102	1.5000 ^{A}	68.70 ^{FGDE}	51.17 ^{BC}
11	KD202	1.5000 ^{A}	43.97 ^{FG}	57.17 ^{BC}
12	OCJ2	1.5000 ^{A}	58.48 ^{FG}	50.50 ^{BC}
13	OCJ9	1.5000 ^{A}	131.92 ^{BC}	51.17 ^{BC}
14	ORO3	1.5000 ^{A}	66.66 ^{FGE}	49.00 ^{BC}
15	ORO9	1.5000 ^{A}	50.15 ^{FG}	61.17 ^{B}
16	YO103	1.5000 ^{A}	102.11 ^{CDE}	50.00 ^{BC}
17	YS103	1.5000 ^{A}	76.10 ^{FDEF}	98.50 ^{A}
18	YS203	1.5000 ^{A}	35.23 ^{GG}	27.00 ^{C}
	Means	n.s	***	**

*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ and ns = not significant

clones based on their mean values for pollen tube length and germination. Clones sharing the same letter are statistically similar in their values, while those with different letters indicate significant differences. The letters are used to indicate the relative performance of each clone, with higher letters indicating better performance in terms of pollen tube length or germination, depending on the column. Among the accessions, EU202 (A) exhibited the highest pollen tube length (177.89 µm), indicating superior pollen viability, while YS203 (G) recorded the shortest (35.23 µm), suggesting lower viability. Similarly, YS103 (A) had the highest germination rate, while YS203 (C) showed the lowest, indicating poorer reproductive potential. Other accessions, such as Brazilian Medium (B) with 137.13 µm and OCJ9 (B) with 131.92 µm, displayed relatively high pollen tube elongation, suggesting better pollen performance under in vitro conditions.

YS103 (98.50%) had the highest success rate for germination, suggesting enhanced pollen viability and reproductive potential, while YS203 (27.00%) had the lowest, implying poor germination capacity. Most accessions clustered within the 50–60% germination range, highlighting moderate viability levels across clones.

DISCUSSION

The assessment of pollen viability and in vitro germination in 18 cashew (*Anacardium occidentale*) clones demonstrated significant variation in reproductive potential, reinforcing the importance of evaluating pollen characteristics for breeding programs. The observed viability percentages ranging from 56.25% to 97.22% align with previous studies indicating genetic and environmental influences on pollen viability and germination capacity [6]. The high viability observed in EU202 (97.22%), CR102 (96.05%), and EN103 (94.17%) suggests these clones possess superior pollen fitness, making them promising candidates for further propagation and hybridization programs. The aniline blue staining technique used in this study has been widely recognized for its reliability in pollen viability assessment [13]. The histochemical staining technique provides a rapid estimate of pollen viability but does not necessarily predict germination success. The differential viability observed among clones is comparable to findings in *Cola nitida*, where staining techniques effectively distinguished viable pollen from non-viable counterparts [6].

The variability in pollen germination across two different media highlights the influence of external factors on pollen performance. Clones such as BEL36, ORO3, YS103, and ORO9 displayed 100% germination in Media 2, suggesting enhanced nutrient availability and osmotic balance in this medium. This observation is consistent with the findings of [13], who noted that sucrose in the germination medium provides essential energy for pollen tube elongation. Additionally, boron and calcium play critical roles in maintaining membrane permeability and strengthening the pollen tube structure, further supporting enhanced germination in optimized media. Interestingly, some clones, including ORO3 and YS203, exhibited no germination in Media 2, while BEL36 failed to germinate in Media 1, indicating genotype-specific responses to media composition. Similar genotype-media interactions have been reported in *Prunus domestica*, where pollen viability and germination rates varied based on the composition of the germination medium [14].

Pollen tube morphology is a key indicator of successful fertilization potential, with straight, elongated tubes being optimal for effective fertilization [15]. The majority of the cashew clones displayed normal pollen tube morphology, reinforcing the favorable reproductive potential of these ac-

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cessions. However, EU202 exhibited abnormal burst tubes (BT), while YO103 showed branching pollen tubes (Br) in Media 2, indicating possible osmotic stress or mechanical resistance to tube elongation. Similar findings were noted by Jayaprakash [15], who reported profuse pollen bursting in liquid media due to rapid hydration, further underscoring the importance of carefully optimized germination conditions. The variation in pollen tube growth direction, with OCJ2, KD102, and India Large showing curvature in Media 1, and BEL36 and YO103 exhibiting curvature in Media 2, suggests differential responses to media-induced stress. Similar findings have been reported by [16], who demonstrated that sucrose concentration influences pollen tube elongation and directional growth in various species.

The significant variations in pollen tube length ($p < 0.001$) and germination percentage ($p < 0.01$) among the clones reinforce the genetic diversity in reproductive performance. The highest pollen tube length recorded in EU202 (177.89 µm) supports its superior pollen viability, while YS203 (35.23 µm) exhibited the shortest tube length, indicative of poor reproductive potential. This aligns with previous studies by Kakani *et al.* [17], which established that a pollen grain is considered germinated when its tube length equals or exceeds its diameter, further supporting the observed differences in viability and germination efficiency. Similarly, the high germination success rate of YS103 (98.50%) compared to YS203 (27.00%) underscores the importance of pollen viability assessment in breeding programs. As reported by [18], some cultivars exhibit sterile pollen or low germination percentages, making pollen characterization crucial for effective cultivar selection.

CONCLUSION

This study emphasizes the importance of pollen viability and in vitro germination for evaluating reproductive potential in cashew clones. Observed variability in these traits highlights the need for genotype-specific optimization of germination media, consistent with findings in other species. Integrating such data into breeding programs can improve parental line selection, fostering the development of superior cashew cultivars and supporting enhanced long-term crop productivity through more effective breeding strategies

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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