



Assessment of Pollen Quality and Germination Dynamics in Chestnut (*Castanea* spp.): Implications of Genotypic Variation and Sucrose Concentration

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Abstract

Pollen viability and germination are critical factors influencing reproductive success and nut yield in chestnuts (*Castanea* spp.). However, their dynamics across different genotypes and cultivars remain insufficient. This study aimed to assess the pollen quality of seven chestnut genotypes/cultivars ('Akyüz,' 'Ali Nihat,' 'Macit 55,' A55, A56, 'Marigoule,' and BDB-L) grown in Samsun, Türkiye. Pollen viability and germination rates were evaluated across four sucrose concentrations (0%, 5%, 10%, and 15%). The results showed significant genotypic differences in pollen quality. The BDB-L genotype consistently exhibited the highest viable pollen percentages (38.48% in 2022 and 40.99% in 2023) and germination rates, peaking at 43.73% in 2023 at 10% sucrose. Similarly, the 'Akyüz' cultivar demonstrated robust performance with viable pollen percentages exceeding 38% across both years and germination rates reaching 40.30% at 5% sucrose. Conversely, A55 and A56 genotypes showed significantly lower pollen viability and germination rates. They also showed the highest non-viable pollen percentages and minimal germination across all sucrose concentrations. The optimal sucrose concentration for pollen germination was determined to be 10% for most genotype/cultivars. These findings underscore the critical influence of genotype and sucrose concentration on pollen quality, providing practical insights for breeding programs and orchard management strategies. The superior performance of the BDB-L genotype and 'Akyüz' cultivar highlights their potential as reliable pollinizers, while the weaker performance of A55 and A56 suggests the need for targeted research to improve their reproductive traits. Future studies should explore the genetic and environmental factors affecting pollen quality to enhance the reproductive success and yield potential of chestnut cultivars.

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1. Introduction

Chestnut (*Castanea* spp.) is a monoecious species characterized by a predominantly self-incompatible reproductive system (Larue et al., 2021; Larue and Petit, 2024). Successful nut production necessitates cross-pollination. The plant displays two separate floral forms: male catkins and mixed-flower catkins (Alinho et al., 2021). Mixed-flower catkins possess female flowers at the terminal of the male flowers. Dichogamy additionally restricts the self-pollination of chestnut trees, as the male and female flowers mature at different times. Consequently, the establishment of orchards requires detailed planning. It is essential to select suitable varieties that have synchronized flowering periods. Understanding the dynamics of pollen quality is essential for optimizing nut yield in chestnut species (Zhou et al., 2023).

The selection of pollinator types that exhibit superior pollen quality is paramount, as viable pollen possessing enhanced germination rates greatly influences the success of fertilization. Pollen quality, including viability, germination, tube growth, and intensely influenced by genotypes and environmental factors, is important for growers, breeders, and research of fertilization biologists (Impe et al., 2020). Moreover, while chestnut pollination has historically been attributed to wind, contemporary studies highlight the predominant role of insects in this process. Consequently, understanding the pollen quality of different species and cultivars is vital for improving pollination and fertilization, which in turn directly impacts nut yield and quality (Larue et al., 2021).

Thirteen chestnut species are found in the northern hemisphere. However, commercial production mainly focuses on three species: *C. sativa* (European chestnut), *C. crenata* (Japanese chestnut), and *C. mollissima* (Chinese chestnut), along with their hybrids (Serdar et al., 2019). Recent years have seen increased interest in hybrid varieties due to the impacts of diseases such as chestnut blight (*Cryphonectria parasitica*), root rot (*Phytophthora* spp.), and the invasive gall

wasp (*Dryocosmus kuriphilus*) (Serdar et al., 2019). These hybrids, created through breeding efforts, seek to merge exceptional nut quality with improved resilience to biotic stresses. As a result, orchards cultivated with these hybrids have emerged as central hubs for production and additional breeding initiatives.

Notwithstanding their increasing significance, the pollen viability and germination rates of numerous hybrid chestnut variations and genotypes are still inadequately comprehended. These characteristics are essential for effective orchard development and breeding operations to enhance cultivars. Although specific studies have examined pollen viability and germination in certain varieties, thorough evaluations across many hybrids are absent.

This study was addressed to assess and compare pollen viability and germination rates among different hybrid cultivars and genotypes grown in Samsun Türkiye ecological conditions. The results will aid in refining orchard management techniques and breeding methodologies, improving nut yield and hybridization initiatives.

2. Material and Method

2.1. Material

This study was conducted in Samsun, Türkiye between 2022-2023. Male flower samples were collected from 7-10 years old chestnut (*Castanea* spp.) plants grown in Ali Nihat Gökyiğit Chestnut Station (41° 23' 53" N, 36° 03' 34" E, 535 m). In the study 7 different hybrid chestnut genotypes were used. 'Akyüz', 'Ali Nihat' and 'Macit 55': In 2004, they were obtained by controlled hybridization of 'King Arthur' (*C. mollissima*/*C. seguine* hybrid) and 'Lockwood' (*C. crenata*/*C. sativa*/*C. dentata* hybrid) varieties at Connecticut Agricultural Research Institute. In 2005, they were imported to Türkiye and included in a selection study. As a result of these studies, 'Akyüz' was selected for its nut characteristics and resistance to the Asian chestnut gall wasp; 'Ali Nihat' was selected for its compact growing characteristics, and 'Macit

55' was selected for its high yield (Macit et al., 2018; Anonymous, 2025).

'Marigoule': This was obtained from a breeding study conducted at INRA France. It is a hybrid of (*C. sativa* var. Migoule (female) x *C. crenata* (male)) (Chapa and Verlhac, 1978). It has a high tolerance to chestnut blight (Hennion, 2010). It was registered as a variety in 2010 because of the studies carried out by Ondokuz Mayıs University to produce certified plants of this variety in our country (Serdar et al., 2011; Anonymous, 2025).

A55 and A56: These genotypes are obtained by the open pollination of the 'Macit 55' cultivar. They stand out with their high yield.

BDB-L: This genotype was introduced to Türkiye from Lebanon as 'Bouche de Betizac'. However, it turned out that it is a hybrid of the 'Bouche de Betizac' cultivar. It produces too many catkins, and they are long, which can be a good alternative for pollination and honey production.

2.2. Method

In this study, viability and the effect of different sucrose concentrations on the germination capacity of chestnut pollen was investigated. During the flowering period, male flowers were monitored and when they were in full blooming they were cut early in the morning and brought to the laboratory in dry cooler basket. The anthers were removed from filaments, and they were placed in the desiccator for 24 hours. After 24 hours, the anthers were sieved through a 100-mesh sieve (0.149 mm) to isolate the pollens.

To determine the viability of the pollens, 1% 2,3,5-triphenyl tetrazolium chloride (TTC) was used (Stanley and Linskens, 1985). A single or double drop of TTC solution was placed on a clean microslide, and pollen grains were dispersed over these droplets using a brush. The drop was meticulously covered with a cover glass, ensuring no air was trapped, and maintained for 2 hours under ambient circumstances. The pollens were subsequently examined using a light microscope at a magnification of 400×. By counting under a microscope, pollen grains stained red were

recorded as alive, those stained pink as semi-alive, and unstained pollen grains as dead (Mert, 2009). For each cultivar/genotype 4 microslides were prepared. For each microslide, 500 pollen grains were counted. A total of 2000 pollen grains were counted per chestnut genotype in this research of pollen vitality.

Measurements of pollen germination were made using techniques outlined by Beyhan and Odabas (1995). Sucrose solutions of varying concentrations were mixed with 1% agar agar. Sucrose concentrations of 0%, 5%, 10%, and 15% were examined. Approximately 10 ml of the medium was dripped into the middle of plastic petri plates (8 mm). Once it cooled and attained a semi-solid state, the pollen grains were spread on the surface of the medium. The petri plates were after covered with parafilm and incubated at 30 °C for 24 hours. Following germination, the pollens in the petri plates were refrigerated until enumeration. Three petri plates were utilized for each sucrose concentration, with six observation sites randomly selected for each plate. The study was conducted using a completely randomized design (CRD) to evaluate pollen viability and germination rates in seven chestnut (*Castanea* spp.) genotypes/cultivars. A minimum of 100–150 pollen grains were counted in each observation field for the germination tests. The germination rate was assessed for each cultivar/genotype. Pollen was deemed germinated if the pollen tube was equal to or exceeded the length of the pollen grain.

All data were analyzed using the R software (2024.09.0, MAC OS). A Shapiro-Wilk test was performed to evaluate the normal distribution of the data. Pollen viability and germination % data were subjected to arcsin transformation prior to statistical analysis. One-way Analysis of Variance (ANOVA) was conducted to assess the effect of sucrose levels. Where significant differences were observed, Duncan's post-hoc test ($P < 0.05$) was applied to determine pairwise differences between the applications.

3. Results and Discussion

In 2022, the analysis of pollen viability across different cultivars/genotypes revealed significant variability in pollen quality parameters. Among the evaluated genotype/cultivars, ‘Akyüz’ cultivar exhibited the highest viable pollen percentage (38.25%), while its non-viable pollen proportion was the lowest (23.76%) (Table 1). Conversely, ‘Ali Nihat’ cultivar showed a balanced distribution between viable (35.36%) and semi-viable pollen (35.76%), with a moderately high proportion of non-viable pollen (28.87%). In

contrast, A55 and A56 genotypes demonstrated the highest non-viable pollen percentages (39.70% and 41.56%, respectively), underscoring their weaker performance in terms of pollen viability. The BDB-L genotype emerged as a standout, showing the highest proportion of semi-viable pollen (44.18%) and the lowest non-viable pollen percentage (17.33%). The ‘Marigoule’ cultivar exhibited moderate performance with a viable pollen percentage of 34.67% and a semi-viable pollen percentage of 39.11%, but its non-viable pollen proportion (26.21%) was higher than BDB-L genotype.

Table 1. Pollen viabilities of the genotype/cultivars tested in 2022

Genotype/Cultivar	Viable	Semi-Viable	Non-Viable
‘Akyüz’	38.25 ± 3.18 a*	37.98 ± 2.31 b	23.76 ± 1.37 c
‘Ali Nihat’	35.36 ± 1.89 a	35.76 ± 1.88 bc	28.87 ± 3.57 b
‘Macit 55’	37.68 ± 3.09 a	33.39 ± 3.33 c	28.92 ± 0.36 b
A55	32.76 ± 3.36 ab	27.53 ± 1.82 d	39.70 ± 3.13 a
A56	29.14 ± 2.83 b	29.29 ± 1.59 d	41.56 ± 1.85 a
BDB-L	38.48 ± 3.38 a	44.18 ± 3.51 a	17.33 ± 0.40 d
‘Marigoule’	34.67 ± 2.52 a	39.11 ± 1.37 b	26.21 ± 3.51 bc
P	<0.05	<0.01	<0.01

*: There is no difference between the means indicated by the same letter in the same column.

The pollen viability assessment for 2023 revealed significant differences among the evaluated genotypes. The BDB-L genotype demonstrated the highest viable pollen proportion (40.99%) with a relatively low non-viable pollen percentage (22.58%) (Table 2). Also, the ‘Akyüz’ cultivar performed well, with a high viable pollen percentage (38.69%)

and the lowest non-viable pollen percentage (20.40%). The ‘Marigoule’ cultivar exhibited moderate viability, with 35.67% viable pollen and a higher proportion of semi-viable pollen (39.17%). ‘Macit 55’ cultivar and A55 and A56 genotypes showed weaker performance, with A56 recording the highest proportion of non-viable pollen (41.45%).

Table 2. Pollen viabilities of the genotype/cultivars tested in 2023

Genotype/Cultivar	Viable	Semi-Viable	Non-Viable
‘Akyüz’	38.69 ± 2.13 ab*	40.90 ± 3.41 a	20.40 ± 3.13 c
‘Ali Nihat’	32.47 ± 2.73 cd	32.04 ± 1.88 de	35.48 ± 0.86 a
‘Macit 55’	30.96 ± 1.89 de	30.18 ± 1.02 e	38.85 ± 1.09 a
A55	28.15 ± 2.58 e	33.90 ± 1.62 cd	37.93 ± 2.97 a
A56	28.46 ± 1.04 e	30.08 ± 1.88 e	41.45 ± 2.91 a
BDB-L	40.99 ± 1.60 a	36.42 ± 3.37 bc	22.58 ± 4.23 bc
‘Marigoule’	35.67 ± 2.17 bc	39.17 ± 0.91 ab	25.15 ± 3.03 b
P	<0.01	<0.01	<0.01

*: There is no difference between the means indicated by the same letter in the same column.

The pollen viability data obtained from 2022 and 2023 indicate significant genotype-dependent changes in pollen quality, underscoring different trends and stability over the years. BDB-L genotype consistently

exhibited superior pollen quality across the genotype and cultivars over both years. In 2023, BDB-L obtained the highest viable pollen percentage at 40.99% while sustaining low non-viable levels at 22.58%, consistent

with its performance in 2022, which showed analogous tendencies. These results are consistent with the work of Beyhan and Serdar (2008), who emphasized the critical role of genotype in determining pollen viability and germination capacity. They reported pollen viability rates of chestnut genotypes typically exceeding 80%, while this study found viability percentages (viable and semi-viable combined) ranging between 58.44% (A56) and 82.67% (BDB-L). The robust performance of BDB-L across both years suggests its potential as a reliable pollinizer. The ‘Akyüz’ cultivar exhibited commendable performance during the two years, regularly ranking among the highest viable pollen and the lowest non-viable percentages. Conversely, A55 and A56 genotypes exhibited a less advantageous trend, characterized by consistently elevated non-viable pollen percentages, especially in 2023, when A56 genotype documented the most significant proportion of non-viable pollen (41.45%). Other genotype/cultivars, like ‘Ali Nihat’ and ‘Marigoule’, exhibited intermediate performance, characterized by fluctuating quantities of viable and semi-viable pollen across the years. These results are in accordance with the results of Altın et al.

(2024). They grouped viability as viable (25.22-40.86%), semi-viable (26.22-43.73%) and non-viable (24.67-37.51%).

In 2022, the pollen germination rates were ranged between 2.71-42.80%, according to the sucrose concentrations (Table 3). Amongst the genotype/cultivars, the BDB-L genotype exhibited superior germination performance across all sucrose concentrations, especially at 10% sucrose (42.80%). This was followed by 15% sucrose (39.46%), highlighting its adaptability and robust pollen viability under varying sucrose conditions. Similarly, the ‘Akyüz’ cultivar performed well, showing a peak germination rate of 40.85% at 5% sucrose and maintaining strong germination rates at 10% sucrose (40.22%). In contrast, A55 and A56 genotypes demonstrated the weakest performance, with significantly lower germination rates across all sucrose concentrations. A55 genotype showed minimal germination, peaking at only 16.90% at 5% sucrose, and consistently underperformed at other concentrations. Although, ‘Macit 55’ cultivar showed moderate germination rates at lower sucrose levels (33.74% at 5% sucrose) and a sharp decline at higher concentrations.

Table 3. Pollen germination rates of genotype/cultivars at different sucrose concentrations in 2022

Genotype/ Cultivar	Sucrose Concentrations (%)				Mean (%)
	0	5	10	15	
‘Akyüz’	4.25 ± 0.23 d*	40.85 ± 0.79 a	40.22 ± 0.88 b	12.85 ± 0.66 d	24.54 ± 0.30 b
‘Ali Nihat’	6.32 ± 0.56 ab	36.45 ± 0.49 b	31.84 ± 1.35 c	17.04 ± 0.30 c	22.91 ± 0.58 c
‘Macit 55’	5.61 ± 0.23 bc	33.74 ± 1.56 c	24.54 ± 0.56 e	10.06 ± 0.23 e	18.49 ± 0.15 d
A55	2.71 ± 0.34 f	16.90 ± 0.36 f	11.98 ± 0.43 f	4.93 ± 0.81 g	9.13 ± 0.37 f
A56	3.36 ± 0.35 e	24.29 ± 1.05 e	28.00 ± 0.37 d	7.19 ± 0.28 f	15.71 ± 0.40 e
BDB-L	6.93 ± 0.10 a	35.05 ± 1.03 bc	42.80 ± 1.80 a	39.46 ± 0.87 a	31.06 ± 0.75 a
‘Marigoule’	5.31 ± 0.46 c	30.05 ± 0.68 d	33.23 ± 0.43 c	22.33 ± 0.45 b	22.73 ± 0.31 c
P	<0.01	<0.01	<0.01	<0.01	<0.01

*: There is no difference between the means indicated by the same letter in the same column.

In 2023, similar results were obtained according to 2022. The pollen germination rate was changed between 3.83-43.73%. According to the cultivars, the highest germination rate was obtained from BDB-L genotype with 33.54%, while the lowest was from A55 genotype with 11.63%. The BDB-L genotype, peaked at 10% sucrose with a germination rate of 43.73%. It also performed exceptionally

well at 15% sucrose (42.14%) and 5% sucrose (40.21%), maintaining consistent and robust germination across varying sucrose levels. The ‘Akyüz’ cultivar also performed well, achieving its highest germination rate at 5% sucrose (40.30%). It also demonstrated a relatively stable performance at 10% sucrose (34.68%). While its germination rate declined at 15% sucrose (14.14%). ‘Akyüz’ cultivar

remained one of the more robust cultivars under lower sucrose concentrations. On the other hand, the lowest germination rates were observed in A55 and A56 genotypes. A55 genotype reached its peak at 5% sucrose (19.21%) but performed poorly at other

concentrations, including 10% sucrose (16.73%) and 15% sucrose (6.77%). Also, A56 genotype exhibited similar trends, with its highest germination rate recorded at 10% sucrose (20.51%).

Table 4. Pollen germination rates of genotype/cultivars at different sucrose concentrations in 2023

Genotype/ Cultivar	Sucrose Concentrations (%)				Mean (%)
	0	5	10	15	
‘Akyüz’	6.40 ± 0.44 b*	40.30 ± 0.98 a	34.68 ± 0.77 b	14.14 ± 0.26 c	23.88 ± 0.12 b
‘Ali Nihat’	3.87 ± 0.61 d	32.82 ± 0.94 b	29.73 ± 0.79 c	19.22 ± 0.47 b	21.41 ± 0.46 c
‘Macit 55’	4.97 ± 0.37 c	28.89 ± 0.45 c	25.12 ± 0.31 d	8.94 ± 1.10 d	16.98 ± 0.38 d
A55	3.83 ± 0.25 d	19.21 ± 1.45 d	16.73 ± 0.99 f	6.77 ± 0.91 e	11.63 ± 0.74 e
A56	4.27 ± 0.33 d	17.49 ± 0.62 e	20.51 ± 0.74 e	6.33 ± 0.41 e	12.15 ± 0.37 e
BDB-L	8.07 ± 0.24 a	40.21 ± 1.20 a	43.73 ± 0.80 a	42.14 ± 0.73 a	33.54 ± 0.56 a
‘Marigoule’	5.24 ± 0.30 cc	28.40 ± 0.60 c	34.67 ± 0.99 b	19.77 ± 0.31 b	22.02 ± 0.54 c
P	<0.01	<0.01	<0.01	<0.01	<0.01

*: There is no difference between the means indicated by the same letter in the same column.

Optimal pollen germination was observed at 10% sucrose for most genotypes, with BDB-L achieving the highest germination rate of 43.73% in 2023. These results closely align with Beyhan and Serdar (2009), who reported germination rates peaking at 43.68% for certain genotypes at the same sucrose concentration. Altın et al. (2024) similarly noted germination rates between 23.01% and 36.63% for most genotypes at 10% sucrose.

The comparatively lower germination rates in A55 and A56, even at optimal sucrose concentrations, parallel observations by Mert and Soylu (2007), who reported significant germination differences among genotypes, particularly in hybrids. This suggests that these genotypes may require targeted breeding interventions or improved cultural practices to enhance their reproductive potential.

4. Conclusion

This study emphasizes the significant role of genotypic variability and sucrose concentration in determining pollen viability and germination dynamics in chestnut (*Castanea* spp.). The BDB-L genotype consistently exhibited superior performance with its highest viable pollen percentages and germination rates across both years. Similarly, the ‘Akyüz’ cultivar demonstrated robust viability and germination, reinforcing its utility

in breeding and orchard management practices. Conversely, A55 and A56 genotypes underperformed, highlighting the necessity for further investigation to address their lower reproductive potential. The findings confirm that a sucrose concentration of 10% optimizes pollen germination across most genotypes. These studies reveal significant insights into the reproductive biology of chestnuts, providing practical recommendations for improving nut yield and breeding methodologies. Future research should investigate the genetic foundations of pollen quality and the influence of environmental conditions to create more resilient and productive chestnut cultivars.

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