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Standardization of pollen collection method and *in vitro* pollen germination media in grapes

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Abstract
The standardization of pollen germination media and pollen collection methods holds significant importance in palynological studies and crop improvement. In this study, among six media combinations studied, media consisting 10% sucrose+100 mg/L boric acid+300 mg/L calcium nitrate resulted in the highest germination rate (71.6%) and could be utilized for further pollen-related studies in grapes. Notably, calcium and boron significantly influenced pollen germination. Additionally, five pollen collection methods were compared, cluster harvested at 50% flowering stage and dried for 24 hours resulted as the most effective method for grape pollen collection, striking a balance between increased pollen quantity and germination percentage (74.9%).

Keywords: Pollen germination, pollen collection, pollen storage, *Vitis*

Introduction
Pollen storage studies mostly aim for their genetic conservation and also for their utilization in crop improvement programme. Pollen storage studies include two important steps namely standardization of the method of pollen collection and standardization of media for *in vitro* pollen germination. The establishment of standardized media for *in vitro* pollen germination is essential to evaluate pollen functionality across various intervals. This standardization is a common practice employed to assess pollen germination, particularly during pollen storage. The primary objective of *in vitro* pollen germination is to replicate *in vivo* conditions as closely as possible. In the laboratory, various parameters need optimization for each plant species, including germination media, temperature, and humidity, to achieve a germination percentage that results in real germination potential (Pinillos and Cuevas, 2008) [1]. Despite the availability of a variety of PGMs, *in vitro* pollen germination experimentation faces challenges. Information regarding PGM requirements is often scattered across diverse publications, making it challenging to compile comprehensive data. Additionally, successful pollen collection ensures the correct estimation of pollen-quality parameters like pollen viability, germination etc., and also ensures the availability of sufficient pollen quantity throughout the storage period which may extend up to a few years. Collection of physiologically mature pollen from open anthers is essential, as immature or old pollen may lack germination capacity, exhibit reduced vigour, or be desiccation-sensitive. Viability decline rates must be studied for specific grape varieties and conditions before collection.

Materials and Methods
In this study, media for *in vitro* pollen germination and method of pollen collection were standardized as a part of the grape rootstock improvement programme. These two studies were preliminary part of "Studies on the pollen micro-morphology, storage behaviours, and cross-compatibility of grape (*Vitis* spp.) genotypes", which was performed on ten grape genotypes from three different grape species (*V. vinifera* L., *V. champini* Planc, *V. parviflora* Roxb.). The pollen of Early Perlette Selection was used for both studies, as it was the earliest in flowering among other genotypes. To standardize media for *in vitro* pollen germination semi-solid media (0.6% agar) with different compositions M1, M2, M3, M4, M5, and M6 (Table 1) were studied. To standardize the method of pollen collection, seven panicles per treatment were bagged with butter paper bags (with fine perforations) prior to collection.

Five different pollen collection methods *viz.* PC1, PC2, PC3, PC4, and PC5 (Table 2) were used for pollen collection and two observations were made i.e., pollen germination on standardized media and quantity of pollens (Fig. 1).

Results and Discussion

The data related to standardization of *in vitro* pollen germination on different media (Table 1) showed that M4 had the highest pollen germination (71.66%) followed by M6 (54.03%) and they differed significantly. M4 and M6 differed only in their sucrose content (10% and 20% respectively). The media compositions M3 and M5 which did not contain calcium nitrate and contained only sucrose and boric acid, had intermediate pollen germination (38.50% and 24.80%). However, M1 (10% sucrose) and M2 (10% sucrose), consisted only sucrose recorded 24.80% and 19.56% pollen germination respectively which is significantly lower than other media combinations. Sucrose, boron, and calcium have their specific roles in pollen germination. Sucrose, an exogenous sugar, plays role in the creation of an osmotic environment and providing nutrition for *in vitro* pollen germination. Lack of sucrose concentration in *in vitro* conditions may lead to pollen bursting (Baloch *et al*., 2001) [2]. Boron is essential for pollen wall structure, sugar absorption, and metabolism, forming a sugar–borate complex that enhances oxygen uptake (Yang and Li, 1999 [3] and Wang *et al*., 2003) [4]. Boron deficiency can lead to pollen tube bursting or failed growth (Fang *et al*., 2019) [5]. Calcium, a central regulator, plays diverse roles in germination through ionic balance and cell signalling (Brewbaker and Kwack, 1963 [6]; Steinhorst and Kudla, 2013 [7]). In *in vitro* germination, calcium is indispensable for growth of the pollen tip, and its deficiency can cause morphological abnormalities in pollen tube (Shivanna and Rangaswamy, 1992) [8]. Since, M4 had proportionate combination of sugar, boron and calcium content, it was superior over other media compositions. The lowest pollen germination in M1 and M2 was due to the fact that they contained only sucrose and lacking in B and Ca.

The selection of a pollen collection method for storage-related studies depends on the delicate balance between the quantity and quality of the pollen. During the standardization of pollen collection methods, in PC1 highest pollen germination (77.7%) was recorded due to freshly collected pollen but collecting the optimum quantity of pollen was limitation (Table 2). In PC2, although the pollen germination rate (74.9%) was lower as compared to PC1, a sufficient quantity of pollen was collected for further studies. In PC3, the quantity of pollen was almost similar to PC2, but the pollen germination rate (44.1%) declined sharply as clusters were dried for 48 hours. Thus, extended drying negatively impacted germination, suggesting an imbalance between quantity and quality. PC1, PC2, and PC3 pollen collection methods were previously used by Sharafi and Bahmani (2010) [9], Kowalczyk *et al*., (2022) [10], and Pereira *et al*., (2018) [11] in grapes. PC4 treatment as followed in apple by Lobaton *et al* (2021) [12] involves vortexing unopened anthers resulting in very low pollen collection as compared to other treatments, also the damaged pollen structure along with some hard-to-separate anther debris was observed under the microscope. Mechanical stress of vortex may have led to deformity of pollen structure thus affecting germination rate. Contrary in PC5 opened flowers subjected to vortex didn't yield any pollen grains quantity indicating absence of pollen in anthers of opened flowers. The choice of pollen collection method significantly influences both the quantity and germination potential of pollen. Drying flower clusters for 24 hours (PC2) appears to strike a balance between increased pollen quantity and maintained germination percentage, making it the best method among those compared.

Table 1: Standardization of media for *in vitro* pollen germination of grapes

S. No.	Media*	Pollen germination (%)
1.	M1: 10% sucrose	24.80^{d} (5.0)
2.	M2: 20% sucrose	19.56^{d} (4.45)
3.	M3: 10% sucrose+100 mg/L boric acid	38.50^{c} (6.2)
4.	M4: 10% sucrose+100 mg/L boric acid+300 mg/L calcium nitrate	71.66^{a} (8.4)
5.	M5: 20% sucrose+100 mg/L boric acid	24.80^{bc} (6.6)
6.	M6: 20% sucrose+100 mg/L boric acid+300 mg/L calcium nitrate	54.03^{b} (7.3)
	LSD ($p \leq 0.05$)	(5.275)

*Semi-solid media solidified using agar (0.6%)

Table 2: Standardization of the various treatments of pollen collection (PC) methods

S. No.	Treatments	Details of the treatments	Observations
1.	PC1	Flower clusters were harvested at 50% flowering stage and used fresh for pollen collection (Sharafi and Bahmani, 2010) [9]	Pollen germination: 77.7% Pollen quantity: Very less quantity of pollen grains was collected as half of the buds were unopened.
2.	PC2	Flower clusters harvested at 50% flowering stage and dried for 24 hours at room temperature followed by pollen collection (Kowalczyk *et al*., 2022) [10]	Pollen germination: 74.9% Pollen quantity: More quantity of pollen grains collected after drying of the cluster.
3.	PC3	Flower clusters were harvested at 50% flowering stage and dried for 48 hours at room temperature followed by pollen collection (Pereira *et al*., 2018) [11]	Pollen germination: 44.1% Pollen quantity: The quantity of pollen was almost similar to PC2.
4.	PC4	Anthers from mature unopened flowers were collected and stored in an Eppendorf tube and dried for 24 hours at room temperature and placed on a vortex machine for 1 minute (Lobaton *et al*., 2021) [12]	Pollen germination: 49.9% Pollen quantity: Less quantity of pollen was extracted and under the microscope damaged pollen grains were observed.
5.	PC5	Fully opened individual flowers were collected and stored in an Eppendorf tube for 24 hours and placed on vortex machine for 1 min.	No pollen grains were harvested.

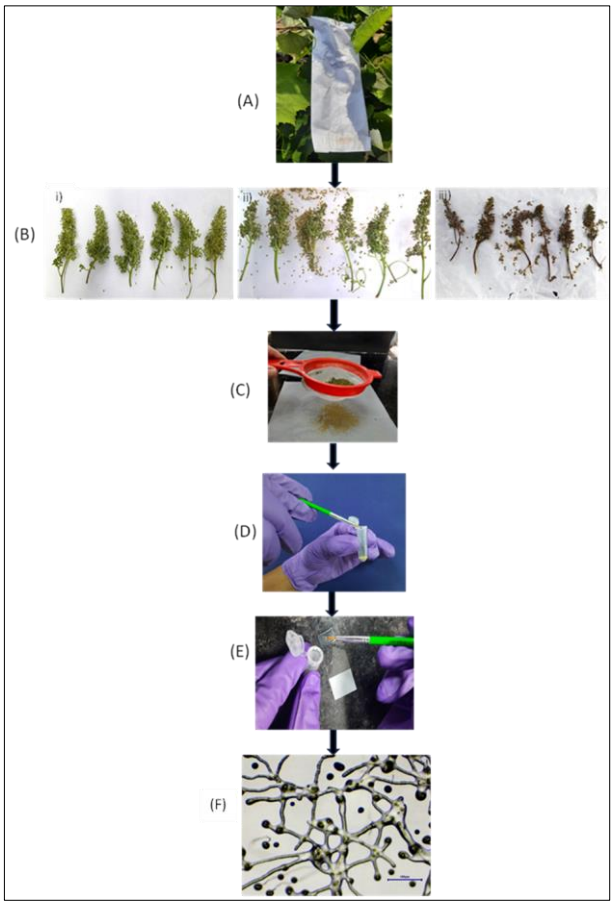

Fig 1: Methodology followed for standardization of pollen collection method: A) Bagging of cluster prior to opening of flowers; B) i.PC1, ii. PC2, iii. PC3; C) Sieving of collected floral matter; D) Transfer of sieved pollen in vials by fine brush; E) Dusting of pollen grains on pollen germination media; F) *in vitro* pollen germination observed under microscope

Conclusion

The study on *in vitro* pollen germination demonstrated that media composition significantly affects pollen germination. The media composition, M4 (10% sucrose+100 mg/L boric acid+300 mg/L calcium nitrate), with a balanced combination of sucrose, boron, and calcium, exhibited the highest germination. The importance of these elements in creating an optimal environment for pollen germination was observed. PC2 (Flower clusters harvested at 50% flowering stage and dried for 24 hours at room temperature followed by pollen collection) emerged as the most effective method, striking a balance between increased pollen quantity and maintained germination percentage. These findings contribute valuable insights for optimizing conditions in pollen-related research and applications.

References

1. Pinillos V, Cuevas J. Standardization of the fluorochromatic reaction test to assess pollen viability. Biotech Histochem. 2008 Jan 1;83(1):15-21.
2. Baloch MJ, Lakho AR, Bhutto H, Solangi MY. Impact of sucrose concentrations on *in vitro* pollen germination of okra, Hibiscus esculentus. Pak. J Biol. Sci. 2001;4(4):402-403.
3. Yang X, Li Y. Boron plays an important role in the regulation of plant cell growth. Tsinghua Sci Technol. 1999 Sep;4(3):1583-1586.
4. Wang Q, Lu L, Wu X, Li Y, Lin J. Boron influences pollen germination and pollen tube growth in *Picea meyeri*. Tree physiol. 2003 Apr 1;23(5):345-51.
5. Fang KF, Du BS, Zhang Q, Xing Y, Cao QQ, Qin L. Boron deficiency alters cytosolic Ca2+ concentration and affects the cell wall components of pollen tubes in Malus domestica. Plant Biol. 2019 Mar;21(2):343-51.
6. Brewbaker JL, Kwack BH. The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot.1963;50(9):859-865.
7. Steinhorst L, Kudla J. Calcium-a central regulator of pollen germination and tube growth. Biochim Biophys Acta Mol Cell Res. 2013 Jul 1;1833(7):1573-81.
8. Shivanna, KR, Rangaswamy, NS. Pollen germination and pollen tube growth *in vitro*, in Pollen biology. Springer, Berlin, 1992, 23-31.
9. Sharafi Y, Bahmani A. Study of pollen germination and tube growth in some Iranian Loquat cultivars and genotypes. In 3rd International Symposium on Loquat 2010 May; 22-25.
10. Kowalczyk BA, Bieniasz M, Kostecka-Gugała A. Flowering biology of selected hybrid grape cultivars under temperate climate conditions. Agriculture. 2022 Apr 30;12(5):655.
11. Pereira MR, Ribeiro H, Cunha M, Abreu I. Comparison of pollen quality in *Vitis vinifera* L. cultivars. Sci. Hortic. 2018 Jan 3; 227:112-6.
12. Lobaton J, Andrew R, Duitama J, Kirkland L, Macfadyen S, Rader R. Using RNA-seq to characterize pollen–stigma interactions for pollination studies. Sci. Rep. 2021 Mar 23;11(1):6635.