



Pollen biology and metaxenic studies on early maturing grape (*Vitis vinifera*) genotypes

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Received: 30 August 2023; Accepted: 19 July 2024

ABSTRACT

The present experiment was conducted during 2020–21 and 2021–22 at ICAR-Indian Agricultural Research Institute, New Delhi to study the impact of open-, self-, and cross-pollination on metaxenic traits (berries) and the incidence of cleistogamy in grapes (*Vitis vinifera* L.). Seven different cross-combinations were used on the grape variety Pusa Navrang, which were planted at 3 m × 3 m spacing and trained on the Y-trellis training system. The experiment was laid out in a randomized block design (RBD) with four replications. The incidence of cleistogamy, berry set, retention percentage, and the metaxenia effect of pollen grains in different grapevine varieties, namely, Pusa Navrang (PN), Black Muscat (BM), Pusa Swarnika (PS), Beauty Seedless (BS), Perlette (PER), Male Hybrid (MH), were studied. Also, pollen quantity and viability were determined for the different grapevine varieties under study. Depending on the genotypes and whether the fruit set is obtained inside the covered flowers, cleistogamy was confirmed. A significantly higher berry set (40–85%) and retention (32–77%) were recorded in open- and self-pollinated flowers. The total number of pollens/anther in six grape genotypes ranged from 7916.62–23832.02, whereas the maximum active pollen rates were noted in the Male Hybrid (70.01%) and Black Muscat (66.80%). The pollens from the Beauty Seedless genotype exhibited significant enhancement of metaxenic traits such as berry set, retention, and size in Pusa Navrang. Therefore, utilizing Beauty Seedless as a pollen parent in grape breeding programmes holds promise for enhancing berry set and overall berry quality, thus enhancing the hybridization efficiency.

Keywords: Pollen parent, Pollination, Pollinizer, Pusa Navrang, *Vitis vinifera*

Grape (*Vitis vinifera* L.) is a highly versatile vine crop that can be grown in various climatic conditions, such as tropical, subtropical, and temperate climates. Grape cultivation has been proven to be one of the most profitable farm enterprises. In India, grapes are cultivated on more than 163,000 hectares, yielding an annual production of 3.401 million metric tonnes and an average productivity of 21.0 metric tonnes/ha (MoAFW 2023). The ‘Pusa Navrang’ is a popular teinturier grape hybrid ideal for cultivation in northern India. It is an early-ripening, basal-bearer variety developed by crossing the Madeleine Angevine and Rubired at the ICAR-Indian Agricultural Research Institute, Pusa, New Delhi, in 1996 (Verma and Usha

2006). However, Pusa Navrang has some drawbacks, such as small berries and significant bold seeds. To enhance its berry characteristics, it is necessary to hybridize it with other grape genotypes that have seedless and larger berries. Genetic variation is necessary to improve specific traits in grapes. Changing pollen parents can improve fruit traits by directly influencing the berry and seed characteristics of the maternal parent through hybridizing. Numerous plant species have undergone extensive study to examine the influence of pollens on the endosperm and embryo, a phenomenon known as xenia, as well as on the surrounding tissues of the embryo, known as metaxenia (Sabir 2011, 2014). Nevertheless, due to grapes’ highly intricate fertilization biology, extensive research is required to achieve successful hybridization and improve grape yield and berry quality.

The present study aimed to characterize and screen pollen parent genotypes for hybridization with Pusa Navrang as the seed parent. In this study, we assessed the efficacy of various pollinizers and their effect on metaxenic traits such as fruit set, fruit retention, and fruit quality components to understand it for different grape genotypes. The primary objectives of this study were to: (i) identify potential occurrences of metaxenia in grapes; (ii) elucidate

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the impact of open-, self-, and cross-pollination on fruit set and retention of Pusa Navrang; and (iii) compare the pollen parents' efficacy with that of a well-known commercial grape variety, while also examining the incidence of cleistogamy in grapes.

MATERIALS AND METHODS

The present experiment was conducted during 2020–21 and 2021–22 at ICAR-Indian Agricultural Research Institute, New Delhi (28° 40' N latitude and 77° 13' E longitude; 228 m amsl). The study utilized a randomized block design (RBD) with four replications. Six-year-old healthy and productive Pusa Navrang vines were planted at 3 m × 3 m and trained Y-trellis system were used for the study. The grapevines received consistent care and maintenance, including pruning, nutrition, irrigation, weed management, and pest and disease control.

Pollination treatments: A total of 28 uniform vines of the Pusa Navrang variety were carefully chosen for the study. This included four vines of each pollinizer genotype, providing a comprehensive selection for the investigation. The study was conducted on four replicates consisting of three panicles on each vine and about 50 flowers on each panicle. The inflorescence panicles were selected based on uniformity, their position on the plant, and four vines for open-pollination. In mid-March, about one week before anthesis, panicles of both Pusa Navrang (PN) and the pollinizer genotypes, such as Black Muscat (BM), Pusa Swarnika (PS), Beauty Seedless (BS), Perlette (PER), Male Hybrid (MH); and Pusa Navrang were covered in cheesecloth frames to avoid out-crossing. Flowers of four similar panicles were allowed to bloom without and within cheesecloth frames for the open- and self-pollinated treatments. About 2–3 days before anthesis, panicles of cross-pollination treatment were emasculated, and 50 stigmas were left in each panicle. The anthers were carefully removed, together with the calyptra, using fine pointed forceps. The emasculated panicles were covered with cheesecloth frames to prevent fertilization from external pollen.

For pollination, the pollens were collected in the evening between 5.00 and 6.00 pm when the calyptra of the first flowers were about to fall and these flowers were dried overnight on butter paper in the laboratory. The following day, pollens were separated from the inflorescence debris by sieving through a fine mesh and stored for two days. The panicles were periodically checked for the appearance of a bead of moisture on the stigma for 2–3 days so that the female parts mature further and attain peak receptivity. During the early morning hours, specifically between 7 am and 9 am, hand-pollination was carried out for each hybrid cross using a paintbrush loaded with designated male pollens that had been previously collected from bagged panicles and stored. After pollination, the stigmas were immediately covered with cheesecloth bags to avoid unwanted cross-pollination. Brushes were used in each cross combination, and the hands of experimenters were sterilized with 70% ethanol between pollinations. One week after the berry

set, the panicles were exposed to full sun throughout fruit development and maturation. The panicles were harvested as they ripened. In each treatment, one panicle was left unpollinated to (i) certify that bags prevented out-crossing with undesirable pollen, (ii) make sure that emasculation was performed at the appropriate time, and (iii) look into the possible degree of cleistogamy.

Cleistogamy incidence, berry set and retention percentage: A series of tests were conducted to determine if the grapevine flowers possess the ability of cleistogamy (Sabir 2014). These experiments involved the removal of the flowering anthers 2–3 days before blooming. The emasculated flowers were then covered with a cheesecloth bag until the fruit set. It was confirmed whether or not each grapevine genotype possessed the characteristic of cleistogamy by observing whether or not the fruit set occurred inside the bagged flowers. The fruit set and the percentage of retained berries that were ultimately harvested were determined by considering the number of pollinated flower count.

Berry analysis: Berries were picked when their total soluble solids (TSS) content ranged from 15–17°B. Mature berries were harvested, counted, and weighed at harvest time for each treatment (Samaan *et al.* 1981). For each sample, the length, width, and weight of 50 berries were examined by digital vernier calipers and a weighing balance, respectively. The volume of the berries was measured by submerging them in a measuring cylinder with water and measuring the volume of the displaced water.

Estimation of pollen quantity and pollen viability: During the peak flowering period, studies were conducted to gather data on pollen. To prevent contamination of pollens from other genotypes, panicles were pre-bagged before the start of the anthesis for each vine. Pollens were collected between 9.00 and 10.00 am by collecting mature flowers in petri dishes. There are three types of flowers in grapevine i.e. staminate (M), pistillate (F), and hermaphrodite (H) flowers. Before anthers dehiscence, 50 anthers were collected from each M and H flower from each genotype, and the pollen quantity was evaluated using a hemocytometer (Neubauer Improved Marienfeld, Germany) as described by Wang *et al.* (2015). Additionally, the viability level of pollens was determined using 1.0% of TTC (2,3,5-triphenyl tetrazolium chloride) for each pollinizer genotype (Wang *et al.* 2015). Pollens were classified based on their colours, such as dark red, light red, and very light pinkish or colourless, and they were further categorized as active, semi-active, and lifeless, respectively (Eti 1991).

Statistical analysis: Data analysis involved univariate ANOVA (analysis of variances), with means compared using LSD (least significant differences). Statistical significance was considered at a level of $P \leq 0.05$ using R-Studio Version 4.2.1 software.

RESULTS AND DISCUSSION

Cleistogamy incidence, berry set and retention percentage: Cleistogamy is a phenomenon in grapevine

flowers where they undergo self-pollination without shedding their calyptra (Sabir 2014). The calyptra is a cap-like structure in the grapevine flower. This process is interesting as it allows the grapevine flower to pollinate itself, which can be advantageous in specific environments. The possible incidence of cleistogamy was estimated for parents utilized in the hybridization programme, in which the flowers were emasculated just before blooming (2–3 days before anthesis) and covered with a cheesecloth bag until the fruit set stage. Depending on the genotypes and whether the fruit set is obtained inside the covered flowers, the cleistogamy was confirmed (Fig. 1). The highest fruit set (68.61%) was recorded for Pusa Swarnika. In contrast, Beauty Seedless was recorded with the least fruit set (42.81%). On a similar note, Staudt (1999) found that 25–35% of 'Muller-Thurgau' and Pinot Noir pollen demonstrated pollen tube growth at the onset of cap fall, indicating early flowering. Conversely, Heazlewood and Wilson (2004) and Sabir (2011) found no evidence of pollen tube growth until the cap fall stage in grapes. This study supports the genotype dependency of this phenomenon, as previously concluded by Vasconcelos *et al.* (2009).

The berry set percentage is considered to be an essential and dependable indicator that measures the level of open, self, or cross-compatibility within and between the maternal genotypes and the pollinizers being evaluated (Samaan *et al.* 1981). This metric helps in understanding the effectiveness of pollination and fertilization processes, which are crucial for successful berry development and yield. The berry set stage plays a crucial role in wine production as it decides the grape yield that can be expected; however, it is essential to note that not all flowers on a vine get fertilized, and the unfertilized ones will eventually drop from the vine (Sabir 2014). The Pusa Navrang variety was used as a female parent in all the combinations, and pooled data for the 2021 and 2022 seasons (Table 1). The berry set was highest in the open- and self-pollination cases (82.4 and 79.11%,

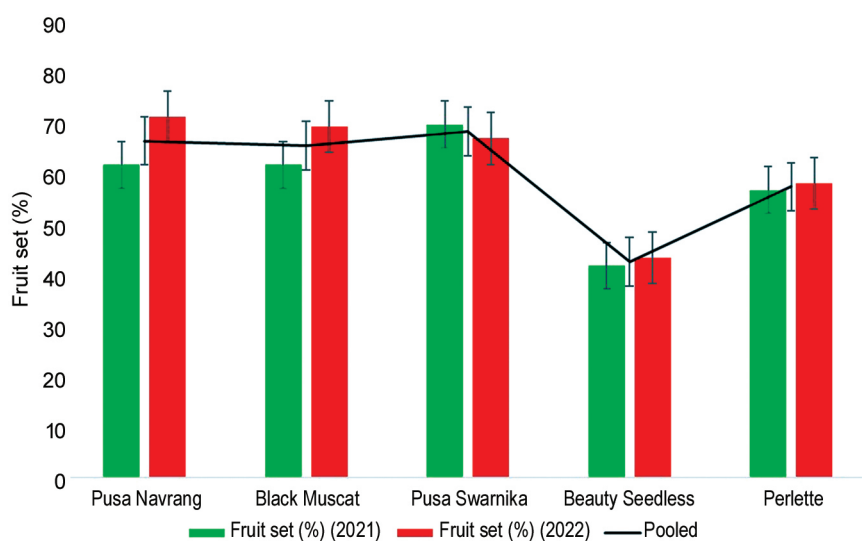


Fig. 1 Percentage of fruit set in grape genotypes to test the cleistogamy in crossing parent genotypes during two consecutive years (2021–22).

respectively). Similarly, fruit retention was the highest (71.84 and 68.95%, respectively) compared to cross-combinations. Among the pollinizers, Pusa Swarnika and Beauty Seedless gave the highest berry set (67.52 and 60.92%, respectively) and retention (55.47 and 51.72%, respectively). On the other hand, Male Hybrid and Perlette showed the lowest berry set (44.15 and 46.33%, respectively) and retention (34.29 and 37.18%, respectively). Several studies involving different grape genotypes have illustrated the diverse effects of pollinizers on berry set (Sabir 2011). In a subsequent study, Sabir (2014) explored the pollinizer capacity of various genotypes and observed the highest berry set in the maternal genotype Narince when pollinated by the Thompson Seedless and Cardinal. According to research into grape fertilization biology, numerous hermaphrodite cultivars, such as Muscat Hambourg (Blidariu and Dobrei 2012) and White Roumi (Samaan *et al.* 1981), have been identified. These cultivars exhibit a preference for cross-pollination to enhance both yield and quality. Sgarbi *et al.* (2010) revealed that Ancellotta was the best pollinizer for Malbo Gentile based on berry set, cluster weight, and flowering time. Marzemino was the least efficient pollinizer for both berry set (26%) and cluster weight at harvest in the grapevine. In the examination of rootstock cross combinations, it was observed that enhanced compatibility led to increased fruit set% and a higher average number of seeds/berry. This was particularly evident in cross combinations such as VP/SC and VP/PN (Rane *et al.* 2024).

Metaxenia effect (Berry characteristics): The influence of different pollen parents on the berry characteristics of Pusa Navrang was studied (pooled data of 2021 and 2022 seasons) (Table 2). Berry shape and size are controlled genetically and typically remain consistent within a cultivar. Nevertheless, pollen genotype affects berry size by influencing hormone production during fruit development (Sabir 2014). When Pusa Navrang was pollinated with Beauty Seedless, it increased berry length, width, volume, and weight (11.99 mm, 12.36 mm, 9.93 mm³, and 9.87 g, respectively) over selfing (10.53 mm, 10.24 mm, 7.18 mm³, and 7.20 g, respectively) and open-pollination (11.09 mm, 11.07 mm, 7.96 mm³, and 8.50 g, respectively). However, it was reduced by Black Muscat, Male Hybrid, and Perlette pollens. Sabir (2014) noted that the characteristics of microsporogenesis can be a significant hindrance in the fertilization process, with self-incompatibility being a rare cause. The influence of pollen from a pollinizer on the maternal plant is categorized as either xenia, which impacts the endosperm and embryo, or metaxenia, which affects the surrounding tissue or berry flesh. Several prior studies have suggested that the size of the fruit (Kumar and

Table 1 Berry set and retention percent in cross-combinations (pooled for 2021 and 2022)

Cross combination	Berry set (%)	Berry retention percent at harvesting (%)
PN × BM	54.43 ^d	44.57 ^c
PN × PS	67.52 ^b	55.47 ^b
PN × BS	60.92 ^c	51.72 ^b
PN × PER	46.33 ^e	37.18 ^d
PN × MH	44.15 ^e	34.29 ^d
PN (self)	79.11 ^a	68.95 ^a
PN (OP)	82.47 ^a	71.84 ^a
LSD (0.05)	5.14	3.88

PN, Pusa Navrang; BM, Black Muscat; BS, Beauty Seedless; PER, Perlette; MH, Male Hybrid; PN(self), Pusa Navrang (self-pollination); PN(OP), Pusa Navrang (open-pollination).

*Distinct letters within the columns denote statistically significant variances as determined by Fisher’s LSD (Least Significant Difference) test at a significance level of $P < 0.05$.

Table 2 Metaxenic effect due to controlled pollination in grape genotypes (pooled for 2021 and 2022)

Cross combination	Berry length (mm)	Berry width (mm)	Berry volume (mm ³)	Berry weight (g)
PN × BM	10.58 ^b	10.30 ^c	6.93 ^d	7.05 ^d
PN × PS	10.84 ^b	11.08 ^b	8.71 ^b	8.86 ^b
PN × BS	11.99 ^a	12.36 ^a	9.93 ^a	9.87 ^a
PN × PER	10.78 ^b	10.30 ^c	6.78 ^d	7.43 ^d
PN × MH	10.94 ^b	10.92 ^{bc}	7.17 ^d	8.28 ^c
PN (self)	10.53 ^b	10.24 ^c	7.18 ^d	7.20 ^d
PN (OP)	11.08 ^b	11.07 ^b	7.96 ^c	8.50 ^{bc}
LSD (0.05)	0.71	0.73	0.53	0.48

PN, Pusa Navrang; BM, Black Muscat; BS, Beauty Seedless; PER, Perlette; MH, Male Hybrid; PN(self), Pusa Navrang (self-pollination); PN(OP), Pusa Navrang (open-pollination).

*Distinct letters within the columns denote statistically significant variances as determined by Fisher’s LSD (Least Significant Difference) test at a significance level of $P < 0.05$.

Das 1996) and the ripening time (Mizrahi *et al.* 2004) are influenced by the pollinizer. Metaxenic effects on fruit size have been previously elucidated, with evidence supporting a decrease in fruit size when smaller-sized cultivars were used as pollen sources (Keulemans *et al.* 1996). However, these findings are contradicted by the results of the current investigation, which found larger berries after pollination with Beauty Seedless, a cultivar known for its relatively smaller berries. The present and earlier research results indicate the possibility of increasing berry size in commercial vineyards using various pollinizer genotypes. During a grapevine pollination experiment conducted by Jovanovic-Cvetkovic *et al.* (2022), it was observed that open pollination yielded the best results. The study also found that pollens from different sources significantly affected bunch and berry weight.

Estimation of pollen quantity and viability: The number of pollens found in anthers across six grape genotypes was examined, which ranged from 7916.62–23832.02 (Fig. 2). In the Male Hybrid genotype, the highest number of pollens were recorded (23832.02). Conversely, the Perlette exhibited the lowest number of pollens (7916.62). This observation aligns with the findings of Kelen and Demirtas (2003), who noted that the Siyah Genre and Razaki varieties had the highest pollen numbers per anther, while the Siyah Demerit and Burdur Dimitri varieties had the lowest. Additionally, a floral biology study by Kowalczyk *et al.* (2022) revealed that pollen grains per anther ranged from 5073–34,976 across various grapevine

cultivars. The number of pollen grains present in the anther can vary considerably due to a variety of factors. These factors include the specific plant species, particular genotype, genetic heritage, and the environmental conditions affecting the process of microsporogenesis process (Kowalczyk *et al.* 2022).

The examination of genotypic pollen viability revealed substantial variability. The results of the TTC test demonstrated that the Male Hybrid and Black Muscat genotypes exhibited the highest rates of active pollen at 70.01 and 66.8%, respectively, while the Beauty Seedless genotype displayed the lowest rate at 34.21%. These findings indicate notable differences in pollen viability among the genotypes (Fig. 3 and Supplementary Fig. 1). The highest semi-active pollen rate was determined in Pusa Navrang (32.39%), and the lowest rate was found in Male Hybrid (7.06%). Genotype Beauty Seedless exhibited

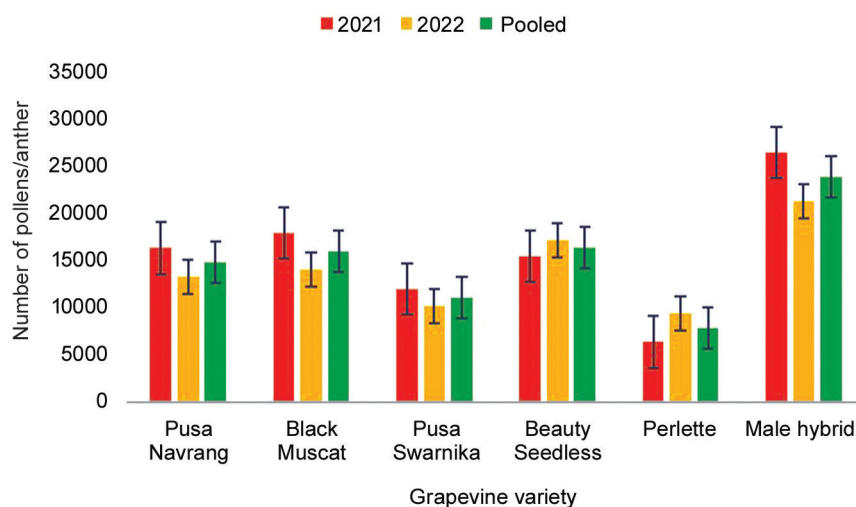


Fig. 2 Pollen quantity in grape parent genotypes used for controlled pollination.

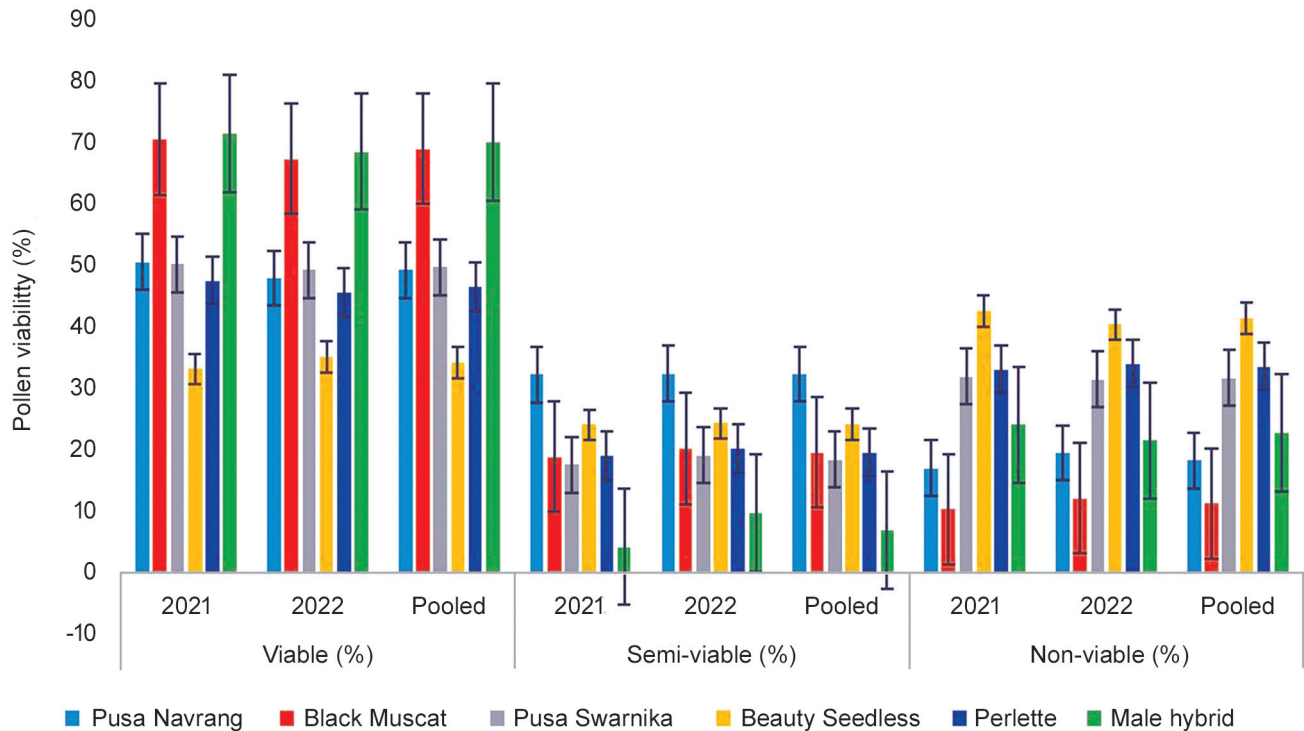


Fig. 3 Pollen viability (%) in selected grape genotypes used as a male parent.

the highest lifeless pollen rate (41.51%), while the Black Muscat genotype had the lowest rate (11.36%). Kelen and Demirtas (2003) reported that varieties Sariemin, Razaki and Siyah Genre had the highest active pollen rates, with the lowest found in the Buzgulu. Additionally, the Siyah Genre and Razaki varieties recorded the highest and lowest semi-active pollen rates, respectively. Further, Kelen and Demirtas (2003) observed that the Buzgulu variety exhibited the highest rate of lifeless pollen, while the Sariemin, Siyah genre, Razaki, and Tilki Kuyrugu varieties displayed the lowest rates. Previous studies examining various genotypes have also revealed diverse pollen viability in grapevines (Sabir 2011, Tello *et al.* 2018, Burcak 2021, Kowalczyk *et al.* 2022, Rane *et al.* 2024).

In the current study, there is notable variation in several berry characteristics in response to different pollen sources, indicating the occurrence of metaxenia in grapes. Consequently, when developing breeding techniques, careful consideration of changing the pollen parents is warranted. The research findings on berry set and various agronomic traits suggest that while the Pusa Navrang variety is well-adapted and self-compatible in North India, its potential genotypes, where higher yield can be realized through cross-pollination with other genotypes. The Male Hybrid genotype exhibited the highest pollen count and pollen viability. Among the pollen parent genotypes, Beauty Seedless emerged as the optimal pollinizer genotype for cultivation in India and other grape-growing regions due to its shorter flowering duration, seedlessness, and high yield in North India. It significantly enhanced specific agronomic traits such as berry set, retention, and size, indicating its

strong pollinating capacity. Additionally, the study of different genotypes revealed significant variability in pollen quality, which can potentially impact pollination efficiency and, consequently, grape productivity. Nevertheless, it is feasible to improve grape productivity by implementing viticulture practices tailored to the unique nutritional needs of each commercial cultivar.

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