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Full Length Article

The Mode of Pollination Influences the Berry Set and Agronomic Features of Table Grape Cultivars (*Vitis vinifera*)

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Abstract

Pollination biology of grapevines displays great diversity ranging from self- to cross-pollination due to rich genotypic variability in flower types. Thus, grape growers and breeders should well-understand the genotype-specific pollination requirements to ensure the food demand of ever-increasing population of the world. A soilless culture was established in protected cultivation to study the possible impacts of self-, hand-, open pollinations and vibration on agronomic features of three table grape cultivars ('Italia', 'Michele Palieri' and 'Prima'). The treatments had different effects on agronomic characteristics of the cultivars, indicating a cultivar-specific pollination response. In 'Italia' and 'Prima' the greatest berry set occurred in hand-pollinated clusters (31.9 and 52.4%, respectively) while vibration resulted in the highest berry set in 'Michele Palieri' (38.5%). In many features, hand pollination provided remarkable improvements in berry, cluster and seed features, indicating the beneficial effects of cross-pollination. Lightness of the berries did not show remarkable variations in response to the different pollination applications across the studied grapevine cultivars. However, chroma in 'Italia' and Hue angle in 'Michele Palieri' were significantly different in open pollination, indicating the diverse effects of pollen sources on berry color, a prime feature of visual quality in grapes. The highest soluble solid contents were found in self-pollination across the cultivars. Overall findings indicated that the pollination treatments significantly affected most agronomic characteristics of the studied cultivars. Thus, viticulturists and breeders should consider the biological response of any cultivar to mode of pollination in grape production or breeding.

Keywords: Grapes; Pollination; Berry set; Agronomy; Xenia

Introduction

Pollination is known as an essential ecosystem service in terms of the quality and yield of fruits and seeds. Due to new market trends on products with functional characteristics and health benefits (Kandylis 2021), grape growers are seeking innovative techniques to increase grape yield and quality. The reproductive features of *Vitis* genotypes display great variation including hermaphrodite, dioecious or polygamous dioic types. Many grape cultivars have self-compatible flowers, while others are self-incompatible, and certain types with complex fertilization biology (Wang *et al.* 2022). A grape inflorescence usually consists of hundreds of flowers. However, the majority of flowers drop in about two weeks after full bloom and normally a small portion of flowers will develop into berries.

Pollination is the essential process for an adequate yield in grapes. Several theories have been proposed about the pollination mechanisms in grapevines, ranging from cross- to self-pollination, according to the existing cultivars, rootstocks and other accessions around the world. Several researchers have investigated adequate fruit set following the natural self-pollination occurrence (Martignago *et al.* 2017), while others have determined simultaneous cross- and self-pollination (Gurasashvili and Vashakidze 2004). Literature reveals certain controversies about the effectiveness of pollen sources and pollination vectors on the quality and quantity of grapes (Staudt 1999; Sampson *et al.* 2001; Sabir 2015), probably due to a wide range of genotypes (Winkler *et al.* 1974) and flower types (Mullins *et al.* 1992) as well as because of a high heterozygosity level in the *Vitis* genome (Martínez-Zapater *et al.* 2010). Several studies concluded that out-crossed pollen from different cultivars significantly increased berry set (Ergul and Marasali 1997; Sabir 2015). Remarkable increases in the number of grape berries were recorded after insect pollination in comparison to bagged flowers (self-pollination) in many hermaphrodite grape cultivars (Sampson *et al.* 2001; Chkhartichvili *et al.* 2006; Sabir 2011). In addition to pollination types, environment and cultivation practices can affect berry set in grapes. The reproductive response of grapevines to environmental conditions can also display great differences. For example, Keller (2015) indicated that certain grape cultivars such as 'Gewürztraminer', 'Grenache' and 'Merlot' were more susceptible to environmental conditions, resulting in insufficient fruit set than others like 'Pinots' and 'Chardonnay'.

In the past, self-pollination (autogamy) was considered to be sufficient for commercial viticulture using hermaphroditic self-compatible cultivars (Winkler *et al*. 1974), because selfing in grapes ensures certain degree of berry set (Mullins *et al*. 1992), if the environmental conditions are favorable. However, ever increasing stress factors, including climate change events, have been limiting productivity in viticulture. Impairments in grapevine physiology and undesirable changes in biochemical reactions have already been detected in response to changes in climatic conditions (Duchêne and Schneider 2005; Orduna 2010).

On the face of multiple stress factors, grape growers have to enhance viticulture practices so as to secure the food needs of an ever-growing population across the world. Protected agriculture, in this context, seems one of the best methods for a sustainable viticulture. Although protected viticulture ensures economic yield in ecologies where frequent extreme environmental conditions restrict grape production, certain issues such as fertilization biology should be considered, as insect activity and wind movement are limited in the protected area (Salvarrey *et al*. 2020; Mahadik *et al*. 2021). Therefore, fertilization biology, which directly influences the yield and quality, should be well-understood to ensure a high income. However, interestingly, there is still inadequate scientific data regarding to the detailed pollination biology features of different grapevine cultivars, except for a few studies indicating the significant positive impacts of pollinizers on the pomological features of maternal cultivars (Sabir 2011; Barbieri *et al*. 2012). Furthermore, the contribution of selfing, insects and wind to berry set and quality in grape species and varieties is not still accurately understood (Sabir and Kucukbasmaci 2020), although there are many studies on the palynology, inflorescence and flower structure of *Vitis* species (Kimura *et al*. 1998; Marasali and Baydar 2001). Recent studies on vineyard conditions revealed that productivity and reproduction in typically self-pollinating grape cultivars were enhanced by cross (by hand) pollination (Sabir *et al*. 2020). Wind was once believed as the most effective pollination factor (Mullins *et al*. 1992). However, wind is theoretically effective on the condition that the insect pollinators are insufficient. Grape pollens are relatively small (30–60 μm in sizes) to maintain buoyant in the air (McGregor 1976). However, airborne pollens are usually prevented by the dense canopy leaves around the grape flower bunches. Besides, the pistils of grape flowers show differences in comparison to most anemophilous genotypes in that they do not have enlarged stigma for more effective intercepting of pollen coming from the air movement. Insects are not necessarily considered essential pollinators of grapes due to their erratic densities in blooming vineyards and the self-fertile character of certain cultivars, although pistillate cultivars such as 'Bicane', 'Cavuş', 'François noir', 'Karagevrek' and 'Maccabéo' require cross pollination.

Therefore, this study was conducted to investigate the effects of different pollination treatments on some agronomic features of three internationally popular table grapes (*Vitis vinifera* L.) grown in soilless culture.

Materials and Methods

Study description

The experiment was conducted at the Research Glasshouse and laboratories of Agriculture Faculty, Selcuk University (38°01.814 N, 032°30.546 E and 1158 m altitude) in Konya, Türkiye. The size of the glasshouse was 3 m in height, 13 m in width and 30 m in length, equipped with two side vents and a flap roof vent. Experimental materials were consisted of three internationally popular *Vitis vinifera* L. table grapes ('Italia', 'Michele Palieri' and 'Prima'). A soilless culture was established in the glasshouse using 70 L black cylindrical pots containing sterile perlite and peat in equal volume to minimize the environmental effects on plant growth and biology. Each cultivar consisted of twelve healthy vines divided into three replicates. Six-year-old experimental vines grafted on 99 R rootstock were placed in east-west oriented lines with 0.5 m and 1.0 m spacing within and between rows, respectively. At the beginning of the vegetative growth prior to the bud break, the study grapevines were spur pruned to leave 8–12 winter buds on 6–8 canes for each grapevine. The summer shoots were fixed to wires set about 2.3 m over the grapevine pots to ensure plants grow in a vertical shape for equally benefited from sunlight. The grapevines all received the same viticulture practices and were watered wit drip irrigation system with a line for each plant row, individual emitter per grapevine. The amount and intervals of irrigation were programmed considering the water matrix potential (Ψm) levels of the growth medium (peat plus perlite) employing tensiometers (The Irrometer Company, Riverside, CA) placed at about 12 cm apart from the trunk with a depth of 20 cm for a long period accurate expression of growth medium water as used by Sabir and Kucukbasmaci (2020). Roof and side vent window area of the experimental glasshouse was large enough, being equivalent to about 25% of floor area, to provide good air movement.

Treatments

Pollination treatments consisted of four applications: i. self-pollination, ii. hand pollination (cross pollination), iii. open pollination and, iv. vibration. Prior to flowering, a total of 48 grape inflorescences for each grape cultivar were labeled according to their experimental groups. Each treatment consisted of three replicates in which four inflorescences were used. To ensure logical evaluation of the treatments, similar sized inflorescences bearing 250–350 buds each were chosen (Samaan *et al*. 1981; Sabir 2011). Just before the summer vegetation period, approximately five days prior to anthesis (Staudt 1999), inflorescences for the self-pollination treatments for each cultivar were covered within cheesecloth frames in order to prevent undesired extraneous cross pollination (Sabir 2015). For the open pollination group, all twelve inflorescences were led to bloom and be pollinated spontaneously. Hand pollination was carried out by brushing the mixture of out-crossed pollen collected from distinct cultivars ('Michele Palieri', 'Alphonse Lavallée' and 'Italia') present inside the glasshouse at the bloom stage. The fresh pollen was harvested by shaking the flowering inflorescences into petri dishes. The inflorescences were controlled daily for the emergence of a moisture bead on the stigma showing that the pistils had matured enough and attained peak receptivity. When the pistils had attained receptivity, pollination was performed in the early morning (08:00–10:00 A.M.) by brushing the pollen onto the functional stigmas. This implementation was repeated for three following days to ensure that all the sequentially opening pistils on the bunch were pollinated (Sabir 2015). The inflorescences belonging to the vibration treatment were gently shaken at anthesis (full bloom) stage for about five seconds by hand between 08:00 and 10:00 A.M. This was also repeated for three consecutive days. One week following the berry set, the cheesecloths on self-pollinated clusters were opened to expose the growing berries to sun light throughout enlargement and maturation period. The inflorescences were tagged at the pollination implementation and were gathered individually when they ripened.

Measurement and analyses

The air temperature and relative humidity between 07:00 and 09:00 A.M. inside the glasshouse at flowering and pollen tube growth stage were recorded with a data logger (EBRO EBI 20). To analyze the physical and chemical characteristics, clusters were harvested when the berries attained the full color stage and the SSC (total soluble solid content) reached at least 15% according to cultivar characteristics Grape clusters of each treatment were gathered, weighed and counted (Samaan *et al*. 1981). Twelve sample clusters within each cultivar were chosen as indicated in the list of international grapevine descriptor catalogue (OIV 1983). The length, width and mass of clusters were obtained in the laboratory. The number of grape berries per cluster was recorded at harvest to calculate the berry set percentages of clusters. Berry length and width of 60 samples for each treatment were measured using digital calipers. Skin color (C; (chroma), h°; Hue angle and L; lightness) of 60 grape berries for each treatment was obtained by a colorimeter Minolta® CR-400 (McGuire 1992). For analysis on berry biochemical features, the sample berries were pressed by hand in cheesecloth to get sufficient grape juice (must). Total soluble solid content (SSC, °Brix) was obtained using a refractometer (Atago 9313). Titratable acidity (TA) was determined by carrying out a titration of 10 mL of the grape juice with 0.1 N NaOH to pH 8.1 and recorded as % tartaric acid. All analyses were carried out in triplicate. The seeds of berries were extracted and washed to extract fruit pulp. Then the seeds were dried for about 2 h with a slight air movement so as to let the dew on seeds evaporate around the room temperature. For seed physical features, 60 seeds per treatment were randomly collected dividing into three replicates and thickness (side view), length and width (front view) were measured with digital calipers. The seed weight was obtained using precision balance and calculated as mg per single seed.

Statistical analysis

Collected data were evaluated with one-way analysis of variance (ANOVA). Differences between the mean values were recorded as significant at $P < 0.05$ and comparison of the means were performed with LSD (least significant difference) test using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

As illustrated in Fig. 1, the air temperatures between 07:00 and 09:00 A.M. at flowering and pollen tube growth stages were between 16.0 (20.04.2020) and 27.3°C (30.04.2020). Air relative humidity recorded at midday (at around 13:00 P.M.) inside the experimental glasshouse ranged from 35.7% (19 April 2018) to 60.0% (20 April 2020). The mean values for day temperature and relative humidity were 23.9°C and 41.8%, respectively.

Satisfactory berry set percentages were achieved in the clusters of the examined grapevine cultivars (Fig. 2), varying from 17.9% (self-pollination in 'Michele Palieri') to 52.5% (hand pollination in 'Prima'). The berry set response of the grapevine cultivars to the pollination treatments showed significant differences between the mean values. Berry set values of hand pollination treatments were significantly higher than those of self-pollination across the cultivars studied, indicating the important contribution of additional pollen of neighboring cultivars. In 'Italia', the berry set varied from 26.5% (vibration) to 35.7% (hand pollination). Besides, the greatest berry set percentage was determined in the vibration treatment (38.5%) in 'Michele Palieri' while the lowest value was recorded from selfing (17.8%). Hand and open pollination in this cultivar resulted in quite low berry set in comparison to vibration. 'Prima' cultivar, with its smaller berries compared to others, set more berries per cluster with a range of 52.5% (hand pollination) to 35.3% open pollination.

Berry weight significantly varied in response to the treatments in all cultivars (Table 1). In 'Italia' the highest berry weight was obtained from vibration while open pollination and self-pollination resulted in the highest berry

Table 1: Variation in berry size and berry weight of grape cultivars in response to various pollination applications

Cultivar	Treatment	Berry weight (g)	Berry width (mm)	Berry length (mm)
'Italia'	Self-pollination	4.06 ± 0.7^{c}	17.20	21.20
	Hand pollination	4.26 ± 1.1^{b}	17.21	20.93
	Open pollination	4.32 ± 0.5^{b}	17.23	20.76
	Vibration	4.76 ± 1.5^{a}	17.43	21.72
	LSD	*0.25*	*ns*	*ns*
'Michele Palieri'	Self-pollination	5.86 ± 0.22^{a}	19.5 ± 0.23^{a}	22.57 ± 0.91^{b}
	Hand pollination	4.44 ± 0.23^{b}	18.16 ± 0.18^{b}	20.67 ± 0.31^{c}
	Open pollination	6.17 ± 0.72^{a}	20.24 ± 0.40^{a}	24.73 ± 0.48^{a}
	Vibration	5.86 ± 0.71^{b}	17.69 ± 0.18^{b}	20.38 ± 0.52^{c}
	LSD	*0.78*	*2.59*	*2.85*
'Prima'	Self-pollination	3.75 ± 2.12^{a}	16.51 ± 0.23^{a}	19.6 ± 0.26^{a}
	Hand pollination	3.40 ± 0.96^{b}	14.78 ± 0.21^{c}	17.72 ± 0.23^{b}
	Open pollination	3.29 ± 0.62^{bc}	15.38 ± 0.23^{b}	17.91 ± 0.18^{b}
	Vibration	3.13 ± 1.38^{c}	14.58 ± 0.13^{c}	16.94 ± 0.14^{c}
	LSD	*0.32*	*1.23*	*1.56*

Means having distinct letters in a column are significantly different according to Student's t-test ($P < 0.05$), ns: not significant

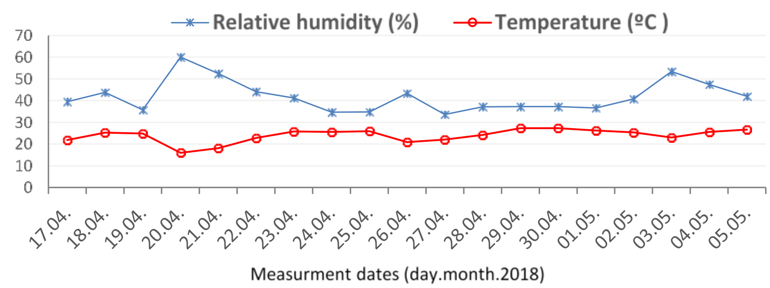

Fig. 1: Relative humidity (%) and temperature (°C) values recorded inside the glasshouse between 07:00 and 09:00 A.M. during the flowering and pollen tube growth period

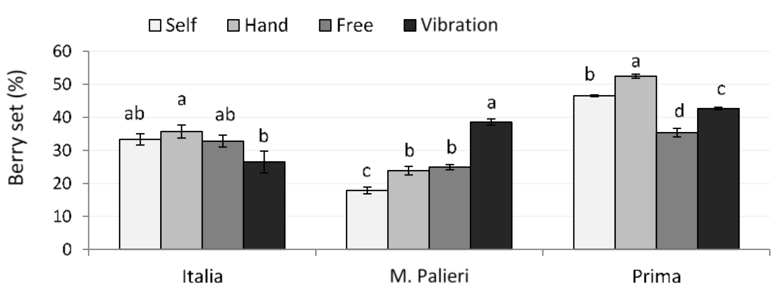

Fig. 2: Variation in berry set (%) of grape cultivars as affected by different pollen sources. Bars with different letter superscripts show significant difference from each other at $P < 0.05$ according to LSD test

weights in 'Michele Palieri' and 'Prima', respectively. Width and length values of the berries displayed similar changes to berry weight with significant variations in 'Michele Palieri' and 'Prima' according to the treatments (Table 1).

As can be seen in Table 2, cluster features such as weight, length and width were generally affected by the treatments. In 'Italia' the highest cluster weight was obtained from hand pollination, followed by vibration and open pollination with different significance levels. On the other hand, open pollination and self-pollination were the most effective treatments influencing cluster weights of 'Michele Palieri' and 'Prima' cultivars, respectively. Open pollination led to remarkable increases in length of the 'Italia' clusters, while self-pollination was predominant with respect to increasing cluster sizes in 'Prima'.

Changes in berry color coordinates in response to various pollination applications are presented in Table 3. Berry lightness (L) did not display significant changes in response to the treatments. However, the treatments had significantly different effects on chroma (C) and Hue angle values of 'Italia' and 'Michele Palieri' cultivars, respectively. The greatest C value was found in hand pollination and was followed by self-pollination and vibration. In comparison, following open pollination the three treatments had similar effects on the intensity of the berry skin color of 'Michele Palieri'. The highest h° value was measured in hand pollination across the cultivars, although significant differences were found in only

Table 2: Variation in cluster size of grape cultivars in response to various pollination applications

Cultivar	Treatment	Cluster weight (g)	Cluster width (cm)	Cluster height (cm)
'Italia'	Self-pollination	204.5 ± 11.5^{b}	9.2	12.8
	Hand pollination	263.4 ± 9.1^{a}	10.2	16.0
	Free pollination	219.4 ± 8.0^{ab}	9.6	14.8
	Vibration	248.6 ± 13.0^{a}	9.2	14.6
	LSD	*40.1*	*ns*	*ns*
'Michele Palieri'	Self-pollination	249.3 ± 6.86^{b}	11.58	15.3 ± 1.23^{b}
	Hand pollination	236.8 ± 6.06^{bc}	9.83	15.9 ± 0.33^{ab}
	Free pollination	273.7 ± 7.63^{a}	11.93	17.6 ± 0.67^{ab}
	Vibration	223.3 ± 14.90^{c}	10.73	17.9 ± 0.20^{a}
	LSD	*16.0*	*ns*	*2.37*
'Prima'	Self-pollination	256.5	15.38 ± 1.40^{a}	21.28 ± 0.89^{a}
	Hand pollination	226.8	13.31 ± 0.50^{ab}	17.71 ± 0.62^{ab}
	Free pollination	238.5	12.30 ± 0.46^{bc}	15.70 ± 2.80^{b}
	Vibration	224.6	10.15 ± 0.70^{c}	18.20 ± 0.98^{ab}
	LSD	*ns*	*2.58*	*3.66*

Means having distinct letters in a column are significantly different according to Student's t-test ($P < 0.05$), ns: not significant

Table 3: Variation in berry color of grape cultivars in response to various pollination applications

Cultivar	Treatment	L	C	h°
'Italia'	Self-pollination	41.9	15.7 ± 0.5^{a}	111.8
	Hand pollination	42.7	16.4 ± 0.3^{a}	113.5
	Open pollination	43.2	12.6 ± 0.4^{b}	109.8
	Vibration	43.4	15.1 ± 0.5^{a}	109.8
	LSD	*ns*	*2.32*	*ns*
'Michele Palieri'	Self-pollination	27.11	1.55	324.6 ± 2.1^{a}
	Hand pollination	27.19	1.82	330.1 ± 2.6^{a}
	Open pollination	26.31	1.55	304.3 ± 6.2^{b}
	Vibration	26.80	1.60	321.5 ± 1.6^{a}
	LSD	*ns*	*ns*	*16.72*
'Prima'	Self-pollination	27.03	1.69	284.7
	Hand pollination	28.28	2.54	290.3
	Open pollination	28.29	2.60	283.9
	Vibration	28.46	2.72	299.9
	LSD	*ns*	*ns*	*Ns*

Means having distinct letters in a column are significantly different according to Student's t-test ($P < 0.05$), ns: not significant. L: Lightness, C: Chroma, h°: Hue angle

Table 4: Variation in biochemical features of grape cultivars in response to various pollination applications

Cultivar	Treatment	SSC	TA	pH
'Italia'	Self-pollination	18.00 ± 0.0^{a}	0.50	3.86 ± 0.01^{a}
	Hand pollination	16.13 ± 0.1^{c}	0.47	3.79 ± 0.05^{b}
	Open pollination	15.10 ± 0.1^{d}	0.53	3.72 ± 0.08^{c}
	Vibration	17.06 ± 0.1^{b}	0.53	3.79 ± 0.01^{b}
	LSD	*0.26*	*ns*	*0.13*
'Michele Palieri'	Self-pollination	16.06 ± 0.2^{a}	0.33	4.21 ± 0.02^{b}
	Hand pollination	14.86 ± 0.1^{b}	0.33	4.23 ± 0.04^{b}
	Open pollination	15.66 ± 0.2^{a}	0.33	4.24 ± 0.02^{b}
	Vibration	14.93 ± 0.2^{b}	0.27	4.35 ± 0.03^{a}
	LSD	*0.29*	*ns*	*0.11*
'Prima'	Self-pollination	17.96 ± 0.25^{a}	0.68	3.59 ± 0.08^{b}
	Hand pollination	17.16 ± 0.20^{b}	0.60	3.37 ± 0.03^{c}
	Open pollination	17.20 ± 0.10^{b}	0.55	3.71 ± 0.05^{a}
	Vibration	17.16 ± 0.10^{b}	0.50	3.76 ± 0.08^{a}
	LSD	*0.26*	*ns*	*0.21*

Means having distinct letters in a column are significantly different according to Student's t-test ($P < 0.05$), ns: not significant. SSC: Soluble solid content, TA: Titratable acidity

'Michele Palieri' cultivar. Open pollination in 'Michele Palieri' resulted in the lowest h° compared with the control, indicating the occurrence of the red to violet color of the berries. These findings indicated that 'Michele Palieri' displayed a sensitivity of color polar parameters to out-crossed pollen sources.

Results of biochemical analyses on grape must (berry juice) are presented in Table 4. Pollination treatments had significant effects on SSC and pH values of the cultivars, although TA did not present significant variation. The highest SSC values were determined in self-pollination across the cultivars, indicating the existence of remarkable

Table 5: Variations in seed sizes of grape cultivars in response to various pollination applications

Cultivar	Treatment	Seed length (mm)	Seed width (mm)	Seed thickness (mm)
'Italia'	Self-pollination	6.25 ± 0.03[b]	3.67 ± 0.11[b]	3.03 ± 0.04[c]
	Hand pollination	6.86 ± 0.09[a]	4.15 ± 0.38[a]	3.36 ± 0.03[a]
	Open pollination	6.80 ± 0.04[a]	4.08 ± 0.25[a]	3.18 ± 0.01[b]
	Vibration	6.24 ± 0.06[b]	3.77 ± 0.45[b]	3.17 ± 0.04[b]
	LSD	*0.29*	*0.30*	*0.16*
'Michele Palieri'	Self-pollination	6.88 ± 0.17[a]	4.20 ± 0.08[a]	3.16 ± 0.03[a]
	Hand pollination	6.32 ± 0.04[b]	3.97 ± 0.04[b]	3.01 ± 0.03[ab]
	Open pollination	6.55 ± 0.12[ab]	4.11 ± 0.07[ab]	2.91 ± 0.07[ab]
	Vibration	6.22 ± 0.07[b]	4.09 ± 0.03[ab]	2.64 ± 0.21[b]
	LSD	*0.510*	*0.27*	*0.53*
'Prima'	Self-pollination	6.88 ± 0.14[a]	3.26	3.26
	Hand pollination	6.32 ± 0.10[ab]	3.30	3.30
	Open pollination	6.55 ± 0.19[ab]	3.28	3.28
	Vibration	6.22 ± 0.07[b]	3.24	3.24
	LSD	*0.619*	*ns*	*ns*

Means having distinct letters in a column are significantly different according to Student's t-test ($P < 0.05$), ns: not significant

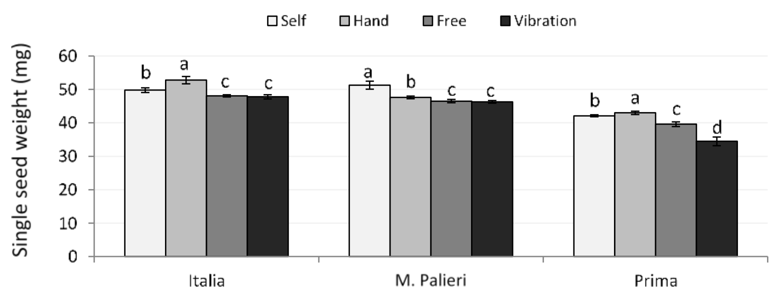

Fig. 3: Variation in seed weights (mg) of grape cultivars as affected by different pollen sources. Bars with different letter superscripts show significant difference from each other at $P < 0.05$ according to LSD test

effects of the pollinizer on the grape ripening process of the maternal cultivar.

As can be seen in Table 5 and Fig. 3, seed features showed significant differences in response to the pollination treatments and the cultivars responded differently to the treatments. To illustrate, the greatest values of length, width, thickness and weight of the seed were obtained from hand pollination in the 'Italia' cultivar, while self-pollinated was the most effective treatment for these parameters in 'Michele Palieri'. In 'Prima' cultivar, the highest seed length was obtained from self-pollination. However, the seed size parameters of self-pollinated berries in 'Italia' were significantly lower than those of both hand and open pollination.

Discussion

It is well-known that environmental factors such as air humidity and temperature directly affect the biology of plants (Stroe *et al.* 2017; Xiao *et al.* 2020). Pollination and berry set incidences in plants are quite sensitive to temperature fluctuations (Stroe *et al.* 2017). The climatic conditions recorded inside the experimental glasshouse were suitable for grapevine palynology and biology since the germination of pollen and pollen tube growth are induced by optimum temperature around 23 ± 4°C depending on variety as indicated by Rajasekaran and Mullins (1985), Staudt (1999), Sabir and Kucukbasmaci (2020). During the flowering and pollination, temperature inside the experimental glasshouse was quite constant without abnormal fluctuation and this allowed us better evaluation of the treatment effects. Berry set evaluation is among the most common and reliable agronomic measures of the intensity of self-, open- or cross-compatibility among the pollinizer and maternal cultivars (Sabir 2011). Satisfactory berry set percentages have been obtained from all the clusters used in the study according to the previous studies conducted on different grape cultivars (Galet 2000; Sampson *et al.* 2001; Sabir 2015; Jovanović-Cvetković *et al.* 2022). It is inferred from the present investigations and previous studies that the percentage of berry set is a genotype-specific feature and pollination types can affect the berry set. Jovanović-Cvetković *et al.* (2022) reported that pollen source affected the berry set of 'Blatina' grape cultivar grown in Bosnia and Herzegovina. In most cases, pollination with pollens from different grape cultivars is more influential e than those of the same genotype's pollen in improving the berry set percentage. In previous studies on the pollination of different grape cultivars Sgarbi *et al.* (2010) and Stroe *et al.* (2017) also found a significant increase in berry set when pollinator cultivars were used. An increase in berry set was also obtained by brushing the pollen of distinct pollinizer genotypes onto the pistils of maternal grapes (Sabir 2011, 2015) and spraying suspension of pollen (Hale and Jones 1956). Furthermore, Sampson *et al*. (2001) revealed that about fifty percent of the berry set in hermaphroditic grape cultivars is emerging from the cross-pollination, verifying the findings of the current study on cross- and open-pollination. Overall, findings on berry set indicated the significance of appropriate pollen movement inside protected agriculture areas. Therefore, artificial pollination support such as proper ventilation, especially in protected cultivation techniques, would be beneficial for satisfactory yield and quality in grape production.

Berry size is one of the prime agronomic characters determining the visual quality and yield of table grapes (Jovanović-Cvetković *et al.* 2022). Therefore, the phenomenon of metaxenia, that is, the influences of the the pollen source cultivar on the berries of the maternal (pollinated) cultivar, should be better understood for both profitable grape production and plant breeding projections. A metaxenic effect on berry size was previously revealed by several researchers (Sabir *et al*. 2015, 2020), although the importance of cross pollination has commonly been ignored by grape growers. The improvement in berry sizes of ‘Italia’ and ‘Michele Palieri’ upon the use of out-crossed pollen in hand or open pollination treatments emphasizes the metaxenial effects in grapevines, as previously indicated by several studies (Martignago *et al*. 2017: Sabir *et al*. 2020).

Berry color is an important quality indicator affecting the consumer acceptance (Abe *et al*. 2007), directly affecting the commercial value of grapes (Liang *et al*. 2009). C is the quantitative feature of colorfulness in grapes. In the present study, hand pollination provided relatively higher C value in ‘Italia’ and ‘Michele Palieri’ cultivars. Higher C indicates high color purity (Ferrara *et al*. 2015), improving the market value of table grapes (Keller 2015) as the berry color becomes more attractive to consumer’s eye (Olivares *et al*. 2017). The greater the C value, the greater is the density of the produce color as distinguished by human eyes. From a marketing viewpoint, treatments essentially affected the visual quality of the grapes. Berry color of red and black grapes is developed by anthocyanin biosynthesis in the grape skins. An improvement in grape skin color coincides with an increase in functional property and an increase in wine quality. Therefore, the treatments might have considerable influence on biochemical and visual features of the grape berries. Hue angle (h°) is the qualitative feature according to which colors have been commonly expressed as greenish, reddish etc. A higher h° means a lesser yellow color feature in horticultural produces. Similar to C value, hand pollination resulted in slightly higher h° in the berries of ‘Michele Palieri’ cultivar.

Biochemical investigations such as SSC, TA and pH in grape juice revealed that pollination treatments affected the ripening of the maternal grape cultivars. Self-pollination resulted in increases of SSC for all the cultivars, probably indicating the delaying effects of pollinizers on the ripening of the maternal cultivar. In Italy, Barbieri *et al*. (2012) also reported significant changes in ripening of ‘Malbo Gentile’ grape cultivar in response to self-, cross- and free-pollination applications, indicating the complex fertilization biology as reported in similar studies on different grape cultivars (Marasali and Baydar 2001; Barbieri *et al*. 2012), probably due to the high heterozygosity level in *Vitis* genome (Martínez-Zapater *et al*. 2010).

Final size of grape berry is known to correlated with the seed features within the berry (Olmo 1946) and the size of the berry is most probably to be a reflection of the success level of fertilization. In the present study, out-crossed pollen remarkably increased the seed sizes in ‘Italia’ similar to the results recorded by Martignago *et al*. (2017) who also found remarkable positive effects of out-crossed pollen on fertilization success in the ‘Bordô’ grape cultivar (*Vitis labrusca* L.). Vibration, on the other hand, was not able to enhance the seed size in comparison to other treatments. It is a well-known fact that berry growth is strongly related to seed development. Such relationship was most probably due to phytohormones such as auxins, cytokinins and gibberellins synthesized in the seeds with a modulating influence of the pollen grain genotype (Zhang *et al*. 2010).

Conclusion

Hand pollination resulted in remarkably higher berry set than selfing in ‘Italia’ and ‘Prima’, indicating a significant contribution of paternal cultivars to berry set. Vibration treatment gave inconsistent results in the cultivars with the greatest berry set in ‘Michele Palieri’ and the lowest berry set in ‘Italia’, while considerably lower berry set in the self-pollination treatment for ‘Michele Palieri’ implied that multi-varietal cultivation should be taken into account to enhance grape quality and yield when ‘Michele Palieri’ is grown. The greatest values in length, width, thickness and weight of the seed were obtained from hand pollination in ‘Italia’ cultivar, indicating the close relationship between seed and berry development, while self-pollination was the most effective treatment for seed parameters in ‘Michele Palieri’. To sum up, the pollination treatments had different effects on many agronomic characteristics among the cultivars, indicating cultivar-specific pollination responses. Therefore, grape growers and breeders should consider the biological response of a given grape cultivar to mode of pollination to enhance the quality features and yield for both open air and protected viticulture.

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Author Contributions

OFB and AS planned and performed the experiment, OFB collected the data, AS carried out statistical analyzing, evaluation of the result and writing the article.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

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