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Two season evaluation of Pinot Noir and Marselan grape hybrids for yield, quality, and biochemical parameters for Chinese grape industry

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This study evaluated the performance of various grape hybrids for yield and quality traits, and to identify hybrid vigor for specific traits that can be utilized in future breeding programs. The research involved evaluating 76 hybrids derived from two diverse parents i.e., Pinot Noir and Marselan, during 2021 and 2022. Multiple fruit yield and biochemical traits were examined and quality traits of peel, flesh, and seeds were recorded. Two-way analysis of variance for parents was performed for all the traits under study to understand the significance of these traits over two years. Likewise, statistical analysis, such as the coefficient of variation, combined transmissibility rate, heterosis, and heterobeltiosis were computed to compare the performance of the hybrids against mid and better parents. The highest CV and Ta values were observed for fruit yield and peel phenolic contents respectively, across both years. The two-year data suggested that certain hybrids displayed superior heterosis and heterobeltiosis for numerous individual traits. Hybrid number 7 exhibited negative hybrid vigor for most of the traits and must be avoided for general cultivation. while hybrid number 25 showed increased hybrid vigor fruit yield and total flavonoids in peel, flesh, and seeds as well as a higher concentration of tannins in seeds. This research underscores the potential of hybrid number 25 for grape farmers and the wine industry in China, hinting at the untapped potential of hybrid grapes in producing superior wines. The study's insights aim to aid the development of indigenous grape varieties, reducing China's reliance on imports and fostering a unique Chinese wine identity in the global market. The data derived from this two-year evaluation can guide future breeding programs and further exploration of grape hybrid vigor to optimize both yield and wine-making quality.
Keywords: Grape breeding, heterosis, heterobeltiosis, juice quality, wine quality.

INTRODUCTION

Grapes (*Vitis vinifera* L.) are among the most widely cultivated fruit crops globally. Breeding grapes with desirable traits and employing advanced techniques are vital for advancing the global grape industry. In China, grape breeding has primarily focused on fruit yield and less attention was given to breed the grapes for wine industry (Atak and Şen, 2021). Hybrid vigor, or heterosis, refers to the superior performance of offspring in comparison to their mid parents, while heterobeltiosis refers to the better performance of offspring in comparison to superior parent. These phenomena are important in hybrid breeding of crops, as these can significantly impact yield in field crops as well as horticultural plants (Qamar *et al.,* 2023). Hybrid breeding in grapes for fruit yield and wine making quality is reported to be a sustainable approach for viticulture industry (Roviello *et al.,* 2021). However, it is not getting as much fame in China as hybrid rice or other crops (Amjad *et al.,* 2022). Consequently, wine grape varieties planted in China are mostly imported from other countries, leading to a shortage of locally produced varieties with superior wine quality (Bitsch, 2021). To foster the development of the grape industry and enhance the competitiveness of Chinese wine products abroad, innovative grape variety breeding to improve wine quality is imperative. Wine grape quality is often linked to the concentration of various substances, such as sugar, acid, phenolic compounds, aroma substances, and

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juice yield (Alessandrini *et al.*, 2018; Jin, 2019; VanderWeide *et al.*, 2020). Understanding the genetics of hybrid vigor for fruit quality can provide a foundation for its enhancement. Previous studies analyzing the offspring of Spanish grape parents and the Longan grape revealed significant heterosis for economically important traits, including fruit weight, shape, and soluble solids (Song *et al.*, 2013). Additionally, analysis of the Longan grape progeny showed that the F_1 generation exhibited heterosis in several economically important traits, such as single fruit weight, fruit shape index, edible rate, and soluble solids (Xu *et al.*, 2015). It was found that different hybrid combinations display unique genetic trends, and a significant additive effect of soluble solids and titratable acid contents was observed in mature fruits of hybrids (Xianming *et al.*, 2002). Grape fruit aroma substances also play a crucial role in overall quality. A wide range of aroma substances were identified in the F_1 generation through the analysis of the cross population between strong aroma and aroma-less varieties, providing ample breeding materials (Guo *et al.*, 2017). In a separate study, Alberto (Hernández-Jiménez *et al.*, 2009) conducted a genetic analysis of anthocyanins in the offspring of the cross between Monastrel and Shiraz, aiming to select new genotypes with quality similar to Monastrel and better suited for different agroecological conditions. In a similar study involving rootstocks, it was found that grafting grapevines onto different rootstocks had a significant impact on grape yield parameters, with some rootstocks resulting in higher yields than others indicating the importance of careful selection of rootstocks for better grape production (Tecchio *et al.*, 2020).

In China, the existing studies have mainly focused on economically important traits like fruit weight, shape, and soluble solids. However, a crucial knowledge gap exists in the evaluation of hybrid grapes in terms of comprehensive wine-making quality parameters, particularly vinification techniques and phenolic substance concentrations in hybrid offspring. This is significant because vinification techniques and phenolic substances are primary indicators of wine quality. The lack of detailed research in this domain means that grape breeders, farmers, and the wine industry in China may not be leveraging the full potential of hybrid grapes to produce superior quality wines. Additionally, understanding these parameters in depth can lead to the development of indigenous grape varieties, reducing reliance on imported varieties and promoting a distinct Chinese wine identity in the global market. Current study focuses on evaluating the basic vinification and phenolic substances from different parts of the fruits of the F_1 generation resulting from the cross between Pinot Noir and Marselan. The objective is to understand the tendency of hybrid vigor to improve grape yield and wine-making quality. To achieve this objective, a total of 76 F_1 crosses between Pinot Noir and Marselan were attempted in 2015 and planted in 2016. It is hypothesized that a two-year evaluation of these hybrids will generate information about the hybrid vigor of these traits which can be useful for farmers and wine industry and can also be used in future breeding programs.

MATERIALS AND METHODS

***Test materials*:** Germplasm was obtained from the grape breeding garden of the Fruit Tree Research Institute within the Shanxi Academy of Agricultural Sciences located at N37.34 °, E112.49 °, and an altitude of (846 ± 5) m on the eastern wing of the Loess Plateau. This region experiences a temperate continental monsoon climate, with an average annual temperature of 10.6°C. The annual rainfall in 2021 was 387.4mm, and the annual rainfall in 2022 was 361.7mm. Seeds were obtained in 2015 by crossing Pinot Noir (a grape genotype having thinner skin and superior wine-making properties) with Marselan (an oldest and better-adapted genotype with a higher skin-to-flesh ratio). In 2016, these seeds were sown at the grape breeding garden, following stratification and other pre-germination treatments. The plants were spaced at 0.5m x 2.5m intervals and subjected to standard management practices. Fruit samples from fully mature parent as well as from hybrid plants were collected during the 2021 and 2022 growing seasons, and subsequently transported to the laboratory in insulated ice boxes for basic quality assessment. Additionally, 100 g aliquots of each fruit sample were flash-frozen in liquid nitrogen and stored at -20°C for subsequent analyses of phenolics, flavonoids, tannins, and proanthocyanidins.

Pinot Noir belongs to the Eurasian species, with early ripening, small ears, tight ears, thin skin, and purple black. Wines made from Pinot Noir are complex and layered, with a smooth, velvety texture. On the other hand, Marselan was obtained by crossbreeding Grenache and Cabernet Sauvignon at the French Agricultural Research Center in 1961. This variety is late maturing, with large ears, moderate tightness, and a blue-black skin. The wine produced is dark in color, rich in fruit aroma, and has a strong sense of structure.

Crossbreeding procedure for hybrid production: Marselan flowers were selected during the early flowering stage and those with several open buds were chosen. The top of the inflorescence was removed and brought indoors after harvesting. Using pointed tweezers, already open flowers were removed, and the inflorescence was placed on a flat and clean white paper. The corollas of the remaining flower buds were removed, and the filaments and ovaries were also removed leaving the anthers behind. The collected pollen was placed in a ventilated indoor area to dry in the shade. The dried pollen was then collected in a clean small glass bottle and the bottle mouth was tightly secured for later use.

Pinot Noir ears that were blooming quickly but had not yet blossomed were selected. The moss cap and pollen sac were gently removed with tweezers, and the entire bouquet was covered with a sealed bag after emasculation to prevent pollination. After 2-3 days of female maturation, water droplets appeared on the stigma. The collected Marselan pollen was dipped onto the Pinot Noir stigma, pollinated completely, and then the paper bag was put back into the bouquet.

The hybrid fruit was specially protected during its growth phase. Fertilizer was applied at the appropriate time, irrigation was done reasonably, and pest control measures were taken. After the fruit was fully ripe, it was harvested, and the seeds were cleaned and stored in layers. The layered seeds were then sown in a small earthen pot for germination in a greenhouse. After the seedlings were formed, they were planted in a breeding garden for normal growth management. Quality identification research was conducted once the results were obtained.

***Quality evaluation of different varieties of wine grape during maturity*:** The grape samples were characterized for basic quality traits according to the Fruit Germplasm Resource Descriptor (Puf, 1990).

***Determination of antioxidant compounds in different parts of fruit*:** Fruit samples were taken from -20°C and their skin, pulp, and seeds were separated. Any remaining juice on the skin and seeds was absorbed using filter paper. Then, 2 g of skin, 6 g of pulp, and 2 g of seeds were separately transferred to 100 mL amber volumetric flasks. Extraction was performed in the dark for 24 hours using 70% ethanol, and the phenolic extracts were filtered. Total phenol and tannin content were determined using the Folin Ciocalteu method (Tian *et al.*, 2011); total flavonoids were quantified by aluminum chloride colorimetry (Tian *et al.*, 2011). Proanthocyanidins content was measured using n-butanol hydrochloric acid colorimetry (Waterhouse et al., 2000). Additionally, 0.4 g of each sample was placed in a 100 mL amber volumetric flask, and extraction was performed in the dark for 12 hours with a 1% hydrochloric acid methanol solution. The resulting filtrate was used as the total anthocyanin extract. Anthocyanin content was determined using the pH differential method, with results expressed as dimethoprim glucoside (Zhen *et al.*, 2004).

***Statistical analysis*:** A two-way analysis of variance was performed in Rstudio 1.0.153 where parents were taken as one factor and year were selected as second factor. Further data analysis and production of chart were performed using boxplot function in Rstudio. Coefficient of variation, genetic transmissibility, and dominance rate calculations were carried out using the following formulas:

Coefficient of variation (CV)=S/ F_1 × 100%

Heterosis = $(F_1 – MP)/MP$

Heterobeltiosis = $(F_1 – BP)/BP$

Combined transmissibility rate Ta= F_1/MP × 100%。

S: standard deviation for F_1, F_1: average value of first generation of crosses, MP = mid-parent value, BP = better parent value.

RESULTS

***Performance of two parents over the periods of two years*:** Significant differences were observed in fruit yield and other traits between the male parent Marselan and the female parent Pinot Noir. Marselan demonstrated higher values for all traits except for average berry weight, skin-to-flesh ratio, and pH of the juice. In contrast, Pinot Noir exhibited significantly higher values for these three parameters. Furthermore, during the 2022 growing season, higher fruit yield, proanthocyanidins content of flesh, total flavonoids content of flesh, total phenolic content of seed, and tannin content of seed were recorded. Additionally, a significant interaction between parent plants and years was observed for titratable acidity of juice, total phenolic content of peel, tannin content of peel, total phenolic content of seed, and total flavonoid content of seed. Notably, an increase in these parameters was recorded for Marselan during 2022 (Table 1).

***Yield parameters for fruit plants*:** The performance of all F_1 grape hybrids were evaluated in comparison to mean as well as better parent and values were recorded as heterosis and heterobeltiosis respectively. Among all studied traits highest coefficient of variation and combined transmissibility rate were recorded for fruit yield and peel phenolic contents respectively. The CV and Ta for fruit yield were 63.20 and 138.90, and 103.75 and 124.01 for the year 2021 and 2022 respectively (Table 2). The heterosis for fruit yield was variable during 2021 and 2022 however, hybrid 13 exhibited the highest heterosis over the two years with a value of 2.43 and the hybrid 55 gave the lowest heterosis with value of -1.93 (Figure S1a, Table S1).

The panicle weight is one of the contributing factors for fruit yield. The CV and Ta for panicle weight were 44.6 and 45.4, and 67.2 and 63.8 for year 2021 and 2022 respectively (Table 2). Hybrid 35 exhibited mild value of heterosis for average panicle weight, with a value of 0.73, representing the highest heterosis. In contrast, hybrid number 7 exhibited the lowest heterosis, with a value of -0.71 (Figure S1b, Table S1). The CV and Ta for berry weight were 27.5 and 28.5, and 110.9 and 112.03 respectively (Table 2). Two years average exhibits that hybrid 73 has highest value (0.86) but mild heterosis for average berry weight. On the other hand, hybrid 49 displayed the lowest degree of heterosis with a value of -0.34. (Figure S1c, Table S1). The CV and Ta for fruit shape index were 6.1 and 6.8, and 97.5 and 96.5 for the years 2021 and 2022 respectively (Table 2). Average two years data indicates hybrid 58 exhibited highest heterosis with the value of 0.24. Conversely, hybrid 63 displayed lowest heterosis with a value of -0.13 (Figure S1d, Table S1). The CV and Ta for skin to flesh ratio were 29.8 and 30.2, and 91.3 and 89.8

Table 1. Mean, standard errors and analysis of variance between two genotypes of different fruit yield and quality parameters of two parents harvested during 2021 and 2022.

Years	2021		2022		***P*-value**
Parents	**Marselan (M)**	**Pinot noir (F)**	**Marselan (M)**	**Pinot noir (F)**	
FY	1955.36±56.3	1539.86±87.2	2225.26±111.9	1638.25±35.0	*p*P***; *p*Y*
APW	119.55±2.93	113.50±3.62	133.58±3.63	113.01±5.24	*p*P**
ABW	1.24±0.05	1.52±0.03	1.22±0.02	1.55±0.01	*p*P***
FSI	1.22±0.03	1.14±0.02	1.24±0.01	1.15±0.02	*p*P**
SFR	0.49±0.02	0.26±0.01	0.50±0.02	0.27±0.01	*p*P***
TSS	22.45±0.44	18.12±0.52	23.16±0.31	18.32±0.25	*p*P***
TA	6.33±0.18	6.92±0.20	6.89±0.12	6.68±0.12	*p*P×Y*
pH	3.46±0.14	3.60±0.10	3.24±0.05	3.56±0.05	*p*P*
JY	53.14±1.81	58.62±1.58	55.34±0.76	56.34±1.36	NS
TPCF	5.27±0.54	4.12±0.07	5.62±0.14	3.96±0.09	*p*P**
TCF	4.54±0.12	3.12±0.06	4.92±0.16	3.24±0.07	*p*P***
PCF	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	*p*P***; *p*Y*
TFCF	0.09±0.001	0.06±0.02	0.09±0.02	0.07±0.02	*p*P***; *p*Y*
TPCP	16.88±0.21	10.80±0.29	18.11±0.25	9.68±0.19	*p*P***; *p*P×Y**
TCP	14.31±0.05	9.51±0.22	15.36±0.33	8.62±0.20	*p*P***; *p*P×Y**
PAP	7.77±0.14	4.07±0.24	8.12±0.21	4.36±0.14	*p*P***
TACP	5.56±0.13	4.53±0.10	5.86±0.15	4.32±0.10	*p*P***
TFCP	9.19±0.21	8.62±0.29	10.68±0.25	7.82±0.19	*p*P***; *p*P×Y**
TPCS	7.39±0.19	5.91±0.33	8.96±0.20	6.24±0.31	*p*P***; *p*Y**; *p*P×Y*
TCS	6.40±0.14	4.99±0.11	7.10±0.18	5.24±0.12	*p*P***; *p*Y**
PCS	0.17±0.01	0.11±0.01	0.18±0.01	0.12±0.03	*p*P***
TFCS	6.21±0.15	2.67±0.13	7.05±0.21	3.11±0.07	*p*P***; *p*P×Y**

*<0.05, **<0.01, ***<0.05 where * depicts significance with 95% probability, ** indicates significance with 99% probability and *** illustrates 99.9% probability. pP= P value of parents effect, pY= P value of year effect, *p*P× Y= P value of parent × year interaction. FY= fruit yield, APW = average panicle weight, ABW = average berry weight, FSI = fruit shape index, SFR = skin-to-flesh ratio, TSS = total soluble solids, TA = titratable acids of juice, pH = pH of the juice, JY = juice yield, TPCF = total phenolic content of flesh, TCF = tannin content of flesh, PCF = proanthocyanidins content of flesh, TFCF = total flavonoids content of flesh, TPCP = total phenolic content of peel, TCP = tannin content of peel, PAP = proanthocyanidins content of peel, TACP = total anthocyanin content of peel, TFCP = total flavonoid content of peel, TPCS = total phenolic content of seed, TCS = tannin content of seed, PCS = proanthocyanidins content of seed, and TFCS = total flavonoid content of seed.

respectively (Table 2). The average of two years heterosis showed that hybrid 13 exhibited highest heterosis with a value of 0.10. On the other hand, hybrid 27 displayed a value of -0.60, indicating the lowest degree of heterosis observed. (Figure S1e, Table S1).

In terms of Heterobeltiosis for fruit yield, hybrid 13 exhibited highest heterosis for two years with average value of 2.14. On the other hand, hybrid 55 displayed the lowest heterobeltiosis with a value of -2.30. (Figure 1a, Table 2).

For heterobeltiosis of panicle weight, hybrid 35 demonstrated the highest two years heterobeltiosis with a value of 0.61, representing mild heterosis. Conversely, hybrid 07 exhibited a value of -0.73, representing the lowest heterobeltiosis. (Figure 1b, Table S2). Likewise, hybrid 73 exhibited the highest value of heterobeltiosis for berry weight (0.68). In contrast, hybrid 49 demonstrated lowest heterobeltiosis with a value of -0.40. (Figure 1c, Table S2). Moreover, for heterobeltiosis of fruit shape index, hybrid 58 exhibited highest value of 1.19. Conversely, hybrid 63 displayed lowest heterobeltiosis with a value of -0.16 (Figure 1d, Table S2). For skin to flesh ratio, the highest degree of heterobeltiosis was observed in hybrid 57, demonstrating a value of 0.63. In contrast, hybrid 27 exhibited a lower degree of heterobeltiosis with a value of -0.69. (Figure 1e, Table S2).

From the above five yield parameters hybrids 21 have shown positive heterosis for four consecutive traits in both years. Similarly, hybrids 13 and 19 have shown positive hybrid vigour for three traits among the five in both years. Whereas hybrids 3, 4, 7, 12, 18, 27, 29, 31, 34, 39, 47, 49, 51, 55, 58, 62, 66, 71, 73 and 75 showed positive heterosis for two traits among the five discussed above.

***Quality parameters for fruit juice*:** Juice yield was having average CV 14.9 and 15.1 and Ta 101.1 and 100.3 for the year 2021 and 2022 (Table 2). For Juice Yield, hybrid 50 exhibited strong heterosis with a value of 5.19, representing the highest degree of heterosis observed. In contrast, hybrid 17 displayed lowest heterosis with a value of -0.47. (Figure S2a, Table S1). For titratable acids, hybrid 72 exhibited mild heterosis with a value of 0.83, representing the highest degree of heterosis observed among all hybrids. On the other hand, hybrid 76

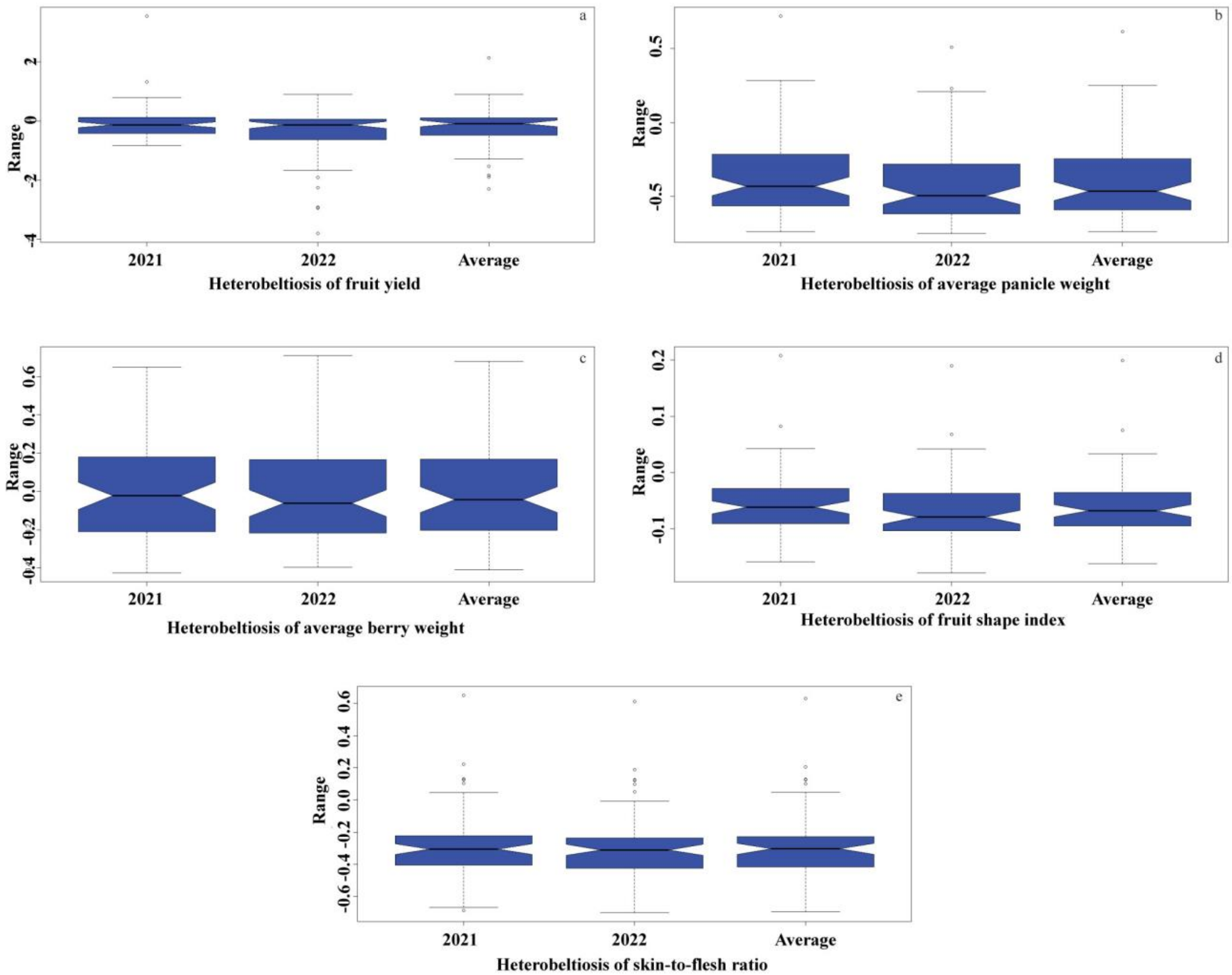

Figure 1. Box plots depict the minimum, maximum, interquartile range and outliers for the heterobeltiosis of yield related parameters of grapes fruits during 2021, 2022 and average of the two years. Figure (a) indicates fruit yield, figure (b) shows panicle weight, figure (c) depicts berry weight, figure (d) explains fruit shape index and figure (e) illustrates skin to flesh ratio.

displayed the lowest degree of heterosis, with a value of -0.71. (Figure S2b, Table S1). For pH, hybrid 67 exhibited slight heterosis, with a value of 0.18, representing the highest degree of heterosis observed among the hybrids studied. In contrast, hybrid 65 displayed the lowest degree of heterosis with a value of -0.10. (Figure S2c, Table S1).

The average CV and Ta for soluble solids ranged from 8.3 and 10.7, and 102.7 and 102.2 respectively (Table 2). Hybrid 26 displayed the highest degree of heterosis, with a value of 0.13, representing a mild level of heterosis. In contrast, hybrid 61 exhibited lowest heterosis with a value of -0.19. (Figure S2d, Table S1).

The averages of two years heterobeltiosis indicate that hybrid 50 exhibited strong heterobeltiosis with a value of 4.99, representing the highest heterobeltiosis among hybrids. In contrast, hybrid 17 displayed lowest heterosis with a value of -0.49. (Figure 2a, Table S2). Similarly, for titratable acids, hybrid 72 exhibited mild heterobeltiosis with a value of 0.77, representing the highest degree of heterobeltiosis observed among all hybrids for two years. On the other hand, hybrid 76 displayed the lowest heterobeltiosis with a value of -0.71. (Figure 2b, Table S2). Moreover, the CV and Ta for titratable acid were 28.2 and 28.9, and 106.2 and 106.5 respectively (Table 2). The average of two years for pH indicates that hybrid 67 exhibited slight heterobeltiosis, with a value of 0.14, representing the highest degree of heterobeltiosis observed among studied hybrids. In contrast, hybrid 65 displayed lowest heterobeltiosis with a value of -0.13. (Figure 2c, Table S2). The average CV and Ta for pH were 5.7 and 8.2, and 1.4 and 6.8 for the years 2021 and 2022 respectively (Table 2). For total soluble solids, hybrid 26 displayed the highest heterobeltiosis for two years with

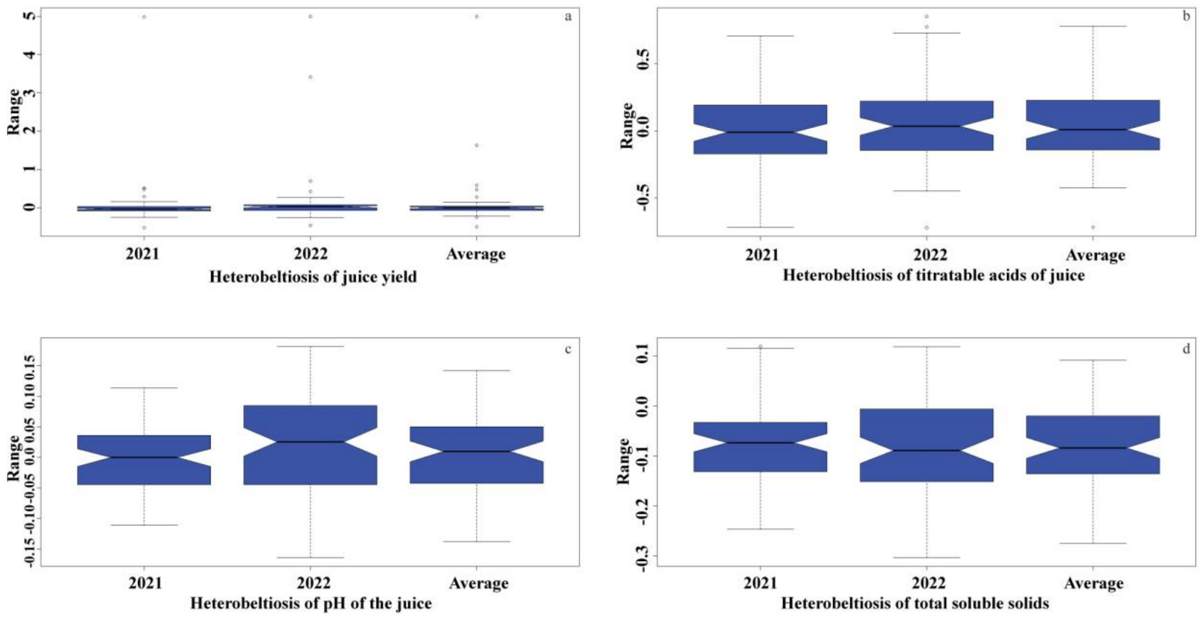

Figure 2. Box plots depict the minimum, maximum, interquartile range and outliers for the het-erobeltiosis of juice related quality parameters of grapes fruits during 2021, 2022 and average of the two years. The horizontal line along x-axis indicating the average values of parents. Figure (a) indicates juice yield, figure (b) shows titratable acid, figure (c) depicts pH value and figure (d) illustrates the soluble solids of fruit juice.

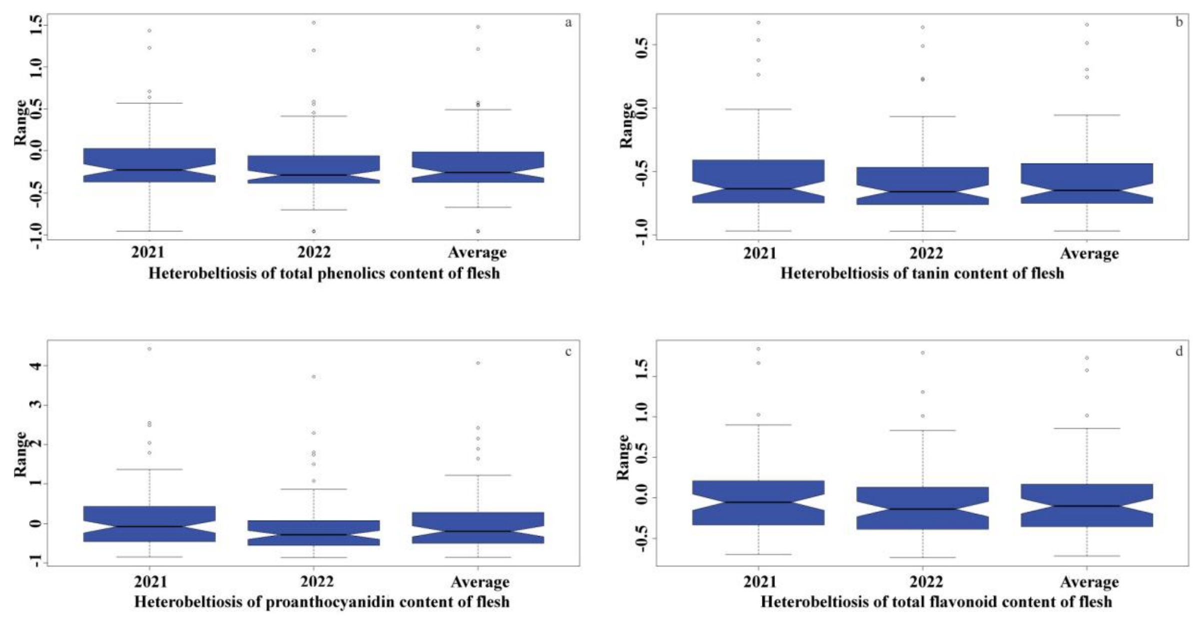

Figure 3. Box plots depict the minimum, maximum, interquartile range and outliers for the heterobeltiosis of quality parameters of fruit flesh during 2021, 2022 and average of the two years. Figure (a) indicates total phenolic contents, figure (b) shows tannin, figure (c) depicts proanthocyanidins and figure (d) illustrates total flavonoids.

average value of 0.11, representing a mild level of heterobeltiosis. In contrast, hybrid 61 exhibited the lowest degree of heterobeltiosis with a value of -0.27. (Figure 2d, Table S2).

For all the four quality parameters of fruit juice, the hybrids 4 have shown positive hybrid vigour. Similarly, the hybrids 2, 8, 16, 19, 35, 51, 55, 59, 66 and 75 have shown positive hybrid vigour for three traits. From the above results it is visible that hybrids 4, 19, 51, 55, 59, 66, and 75 have shown better hybrid vigour results for both fruit juice quality parameters as well as fruit yield parameters making them a good fit for future breeding program.

***Quality parameters for fruit flesh*:** The CV and Ta for total phenolic contents were 47.6 and 49.2, and 97.3 and 96.2 for the year 2021 and 2022 respectively (Table 3).

Table 2. Coefficient of variation, and combined transmissibility rate of fruit yield and fruit juice related parameters of 2021 and 2022.

Index	Years	CV/%	Ta/%
Plant yield	2021	63.20	103.80
	2022	138.90	124.00
Average panicle weight	2021	44.60	67.20
	2022	45.40	63.80
Average berry weight	2021	27.50	110.90
	2022	28.50	112.30
Fruit shape index	2021	6.10	97.50
	2022	6.80	96.50
The skin to flesh ratio	2021	29.80	91.30
	2022	30.20	89.80
Soluble solids	2021	8.30	102.70
	2022	10.70	102.20
Titratable acid	2021	28.20	106.20
	2022	28.90	106.50
pH value	2021	5.70	101.40
	2022	8.20	106.80
Juice yield	2021	14.90	101.10
	2022	15.10	100.30

Table 3. Coefficient of variation, and combined transmissibility rate of fruit flesh quality related parameters of 2021 and 2022.

Flesh Index	Year	CV/%	Ta/%
Total phenolics contents	2021	47.6	97.3
	2022	49.2	96.3
Tannin content	2021	70.5	54.5
	2022	72.5	51.7
Proanthocuanidin content	2021	68.3	117.4
	2022	71.5	103.7
Total anthocyanins content	2021	51.0	113.0
	2022	51.6	108.1

Hybrid 69 exhibited slight heterosis with a two years average value of 1.84, representing the highest degree of heterosis observed among studied hybrids. In contrast, hybrid 26 displayed lowest degree of heterosis with a value of -0.95. (Figure S3a, Table S1). For tannins, hybrid 71 exhibited mild heterosis with a two-year average value of 0.98, representing the highest degree of heterosis observed among the studied hybrids. In contrast, hybrid 28 displayed a degree of heterosis with a value of -0.96, representing the lowest observed value (Figure S3b, Table S1). For proanthocyanidins, hybrid 2 exhibited strong heterosis with a two year average value of 4.69, representing the highest degree of heterosis observed among the hybrids studied. On the other hand, hybrid number 7 displayed the lowest degree of heterosis with a value of -0.83. (Figure S3c, Table S1). Similarly, for total flavonoids hybrid 71 demonstrated considerable heterosis with a two years average value of 2.14, representing the highest degree of heterosis observed among all 76 hybrids. In contrast, hybrid 31 displayed the lowest degree of heterosis with a value of -0.67. (Figure S3d, Table S1).

For heterobeltiosis of total phenolic compounds in flesh, hybrid 69 exhibited slight het-erobeltiosis with a two years average value of 1.47, representing the highest heterobeltiosis among studied hybrids. In contrast, hybrid number 25 displayed lowest value of heterobeltiosis with a value of -0.95. (Figure 3a, Table S2).

For tannins, hybrid 71 exhibited mild heterobeltiosis with a value of 0.65, representing the highest heterobeltiosis among the studied hybrids. In contrast, hybrid 28 displayed a lowest heterosis with a value of -0.97 (Figure 3b, Table S2). The CV and Ta for tannin contents for the year 2021 and 2022 were ranged from 70.5 and 72.5, and 54.5 and 51.7 respectively (Table 3). For proanthocyanidins the average of two years indicates that hybrid 2 exhibited strong heterobeltiosis with a value of 4.07, representing the highest heterobeltiosis among the studied hybrids. On the other hand, hybrid number 7 displayed the lowest degree of heterobeltiosis with a value of -0.85. (Figure 3c, Table S2). Similarly, for total flavonoids hybrid 71 demonstrated considerable heterobeltiosis with a value of 1.72, representing the highest heterobeltiosis among all 76 hybrids. In contrast, hybrid 31 displayed the lowest heterobeltiosis with a value of -0.71. (Figure 3d, Table S1).

The hybrids 68 and 71 have shown positive heterosis for all the four quality parameters of fruit flesh. Similarly, hybrids 34, 38, 72, and 73 showed positive heterosis for three out of four trait parameters.

Table 4. Coefficient of variation, and combined transmissibility rate of fruit peel quality related parameters of 2021 and 2022.

Peel Index	Year	CV/%	Ta/%
Total phenolics content	2021	47.9	132.6
	2022	46.1	131.2
Tannin content	2021	51.9	108.2
	2022	52.0	109.5
Proanthocyanidins content	2021	78.2	95.8
	2022	78.9	91.8
Total anthocyanins content	2021	42.2	88.0
	2022	44.0	88.2
Total flavonoids	2021	56.8	75.7

2022	57.1	60.1

***Quality parameters for fruit peel*:** The CV and Ta for total phenolic contents were 47.9 and 46.1, and 132.6 and 131.2 for the year 2021 and 2022 respectively (Table 4). Hybrid 62 exhibited slight heterosis with a value of 1.73, representing the highest degree of heterosis observed among all hybrids. In contrast, hybrid number 7 displayed lowest heterosis with a value of -0.83. (Figure S4a) (Table S1).

The CV and Ta for tannin contents were 51.9 and 52.0, and 108.2 and 109.5 for the year 2021 and 2022 respectively (Table 4). Hybrid 62 exhibited mild heterosis with a value of 1.29, representing the highest two years average heterosis among studied hybrids. In contrast, hybrid 8 displayed lowest heterosis with a value of -0.82. (Figure S4b, Table S1). The CV and Ta for proanthocyanidins contents were 78.2 and 78.9, and 95.8 and 91.8 for the year 2021 and 2022 respectively (Table 4). Hybrid 62 exhibited mild heterosis with a value of 2.12, representing the highest average heterosis for two years. In contrast, hybrid 36 displayed lowest degree of heterosis with a value of -0.95. (Figure S4c, Table S1). The CV and Ta for total flavonoids contents were 56.8 and 57.1, and 75.7 and 60.1 for the year 2021 and 2022 respectively (Table 4). Hybrid 62 exhibited slight heterosis with a value of 0.60, representing a higher average heterosis observed among studied hybrids. In contrast, hybrid number 7 displayed lowest hybrid vigor with a value of -0.84. (Figure S4d, Table S1). The CV and Ta for total anthocyanins contents were 42.2 and 44.0, and 88.0 and 88.2 for the year 2021 and 2022 respectively (Table 3). Two years average exhibits that hybrid 28 showed highest heterosis with a value

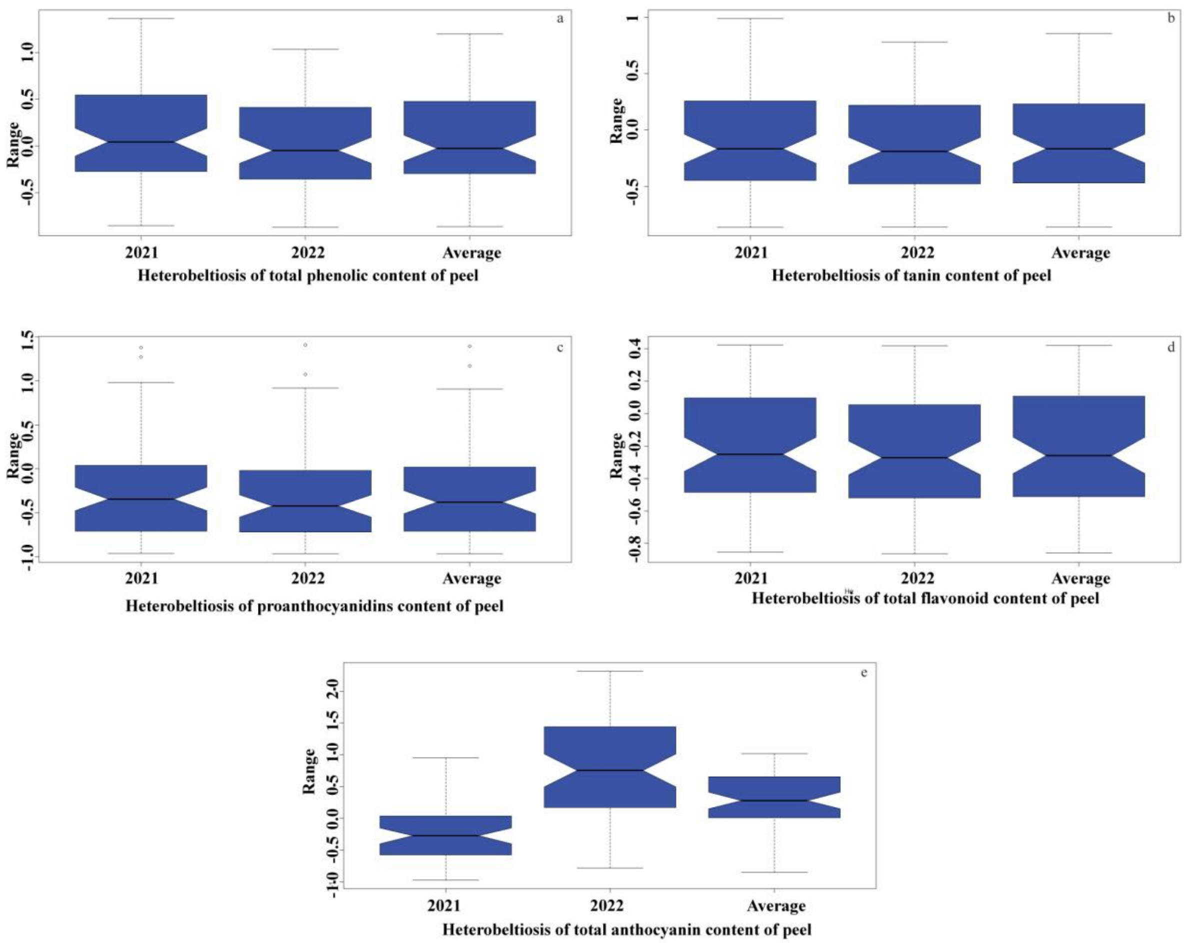

Figure 4. Box plots depict the minimum, maximum, interquartile range and outliers for the heterobeltiosis of quality parameters of fruit peel during 2021, 2022 and average of the two years. Figure (a) indicates total phenolic contents, figure (b) shows tannin, figure (c) depicts proanthocyanidins, figure (d) illustrates total flavonoids and figure (e) explains total anthocyanins.

of 1.27. In contrast, hybrid number 7 displayed lowest values of heterosis i.e., -0.83 (Figure S4e, Table S1).

The average heterobeltiosis of two years for total phenolic contents indicates that hybrid 62 exhibited slight heterobeltiosis with a value of 1.20. It is also representing the highest heterobeltiosis among studied hybrids. In contrast, hybrid number 7 displayed lowest heterosis with a value of -0.86. (Figure 4a, Table S2). Similarly, average heterobeltiosis of tannin indicates hybrid 62 exhibited mild heterobeltiosis with a value of 0.85, representing the highest value of heterobeltiosis among studied hybrids. In contrast, hybrid 8 displayed lowest heterobeltiosis with a value of -0.85. (Figure 4b, Table S2).

For proanthocyanidins, again hybrid 62 exhibited mild heterobeltiosis with a value of 1.39, representing the highest degree of heterobeltiosis. In contrast, hybrid 36 displayed lowest degree of heterobeltiosis with a value of -0.96. (Figure 4c, Table S2). Moreover, again average heterobeltiosis of two years indicates that hybrid 62 exhibited slight heterobeltiosis with a value of 0.42, representing a higher degree of heterobeltiosis among studied hybrids. In contrast, hybrid number 7 displayed lowest hybrid vigor with a value of -0.85. (Figure 4d, Table S2). Moreover, for heterobeltiosis of total anthocyanins, Hybrid 28 exhibited mild heterosis with a value of 1.01, representing a higher degree of heterosis observed among all hybrids. In contrast, hybrid number 7 displayed lowest heterosis with a value of -0.85. (Figure 4e, Table S2).

The hybrids 12, 16, 26, 27, 28, 45, 50, 55, 57, 59, 60 have shown positive hybrid vigor for all the five quality traits of fruit peel. Similarly, hybrids 11, 21, 25, 54, 61, and 62 showed positive hybrid vigour for four traits among the five.

***Quality parameters for fruit seed*:** The CV and Ta during 2021 and 2021 for phenolic contents were 76.1 and 76.3, and 263.8 and 234.1 respectively (Table 5). The average of two years indicates that hybrid 56 exhibited strong heterosis with a value of 7.10, representing the highest degree of heterosis among studied hybrids. In contrast, hybrid 33 displayed lowest degree of heterosis with a value of -0.88. (Figure S5a, Table S1).

Table 5. Coefficient of variation, and combined transmissibility rate of fruit seed quality related parameters of 2021 and 2022.

Seed Index	Year	CV/%	Ta/%
Total phenolics contents	2021	76.1	263.8
	2022	76.3	234.1
Tannin content	2021	79.3	281.1
	2022	79.2	265.7
Proanthocyanidins content	2021	76.5	288.8
	2022	77.2	263.8
Total flavonoid content	2021	84.1	167.7
	2022	83.8	148.0

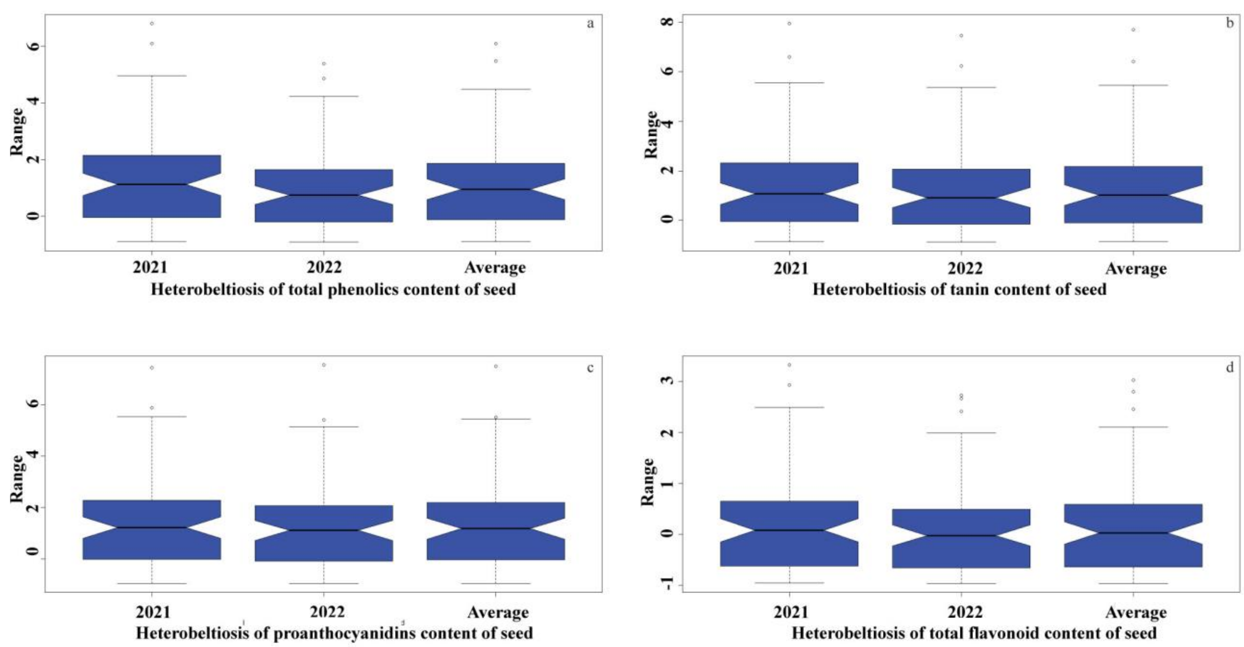

Figure 5. Box plots depict the minimum, maximum, interquartile range and outliers for the heterobeltiosis of quality parameters of fruit seed during 2021, 2022 and average of the two years. Figure (a) indicates total phenolic contents, figure (b) shows tannin, figure (c) depicts proanthocyanidins and figure (d) illustrates total flavonoids.

For heterosis of tannins, hybrid 56 exhibited strong heterosis with two-year average value of 8.90, representing the highest degree of heterosis observed. On the other hand, hybrid 34 displayed lowest degree of heterosis with a value of -0.88. (Figure S5b, Table S1). The CV and Ta during 2021 and 2022 for proanthocyanidins were 76.5 and 77.2, and 288.8 and 263.8 respectively (Table 5). Hybrid 52 exhibited strong heterosis with a two years average value of 9.27, representing the highest degree of heterosis observed among the hybrids studied. In contrast, hybrid 35 displayed lowest degree of heterosis with an average value of -0.94 (Figure S5c, Table S1). Additionally, average two years heterosis of total flavonoids shows that hybrid 56 exhibited highest heterosis with a value of 4.61. In contrast, hybrid 33 displayed lowest heterosis with a value of -0.94. (Figure S5d, Table S1).

The average of two years heterobeltiosis of phenolic contents indicates that hybrid 56 exhibited highest heterosis with a value of 6.10. In contrast, hybrid 33 displayed lowest degree of heterosis with a value of -0.90. (Figure 5a, Table S2). For heterobeltiosis of tannins, hybrid 56 exhibited strong heterosis with a two-year average value of 7.70. On the other hand, hybrid 34 displayed lowest heterobeltiosis with a value of -0.86. (Figure 5b, Table S2). The two years heterobeltiosis of proanthocyanidins exhibits that hybrid 52 showed strong heterobeltiosis with a value of 7.50. In contrast, hybrid 35 displayed lowest degree of heterosis with a value of -0.95 (Figure 5c, Table S2). Moreover, two years average of heterobeltiosis for total flavonoids indicates that hybrid 56 exhibited highest heterobeltiosis with a value of 3.02. In contrast, hybrid 33 displayed lowest heterobeltiosis with a value of -0.95 (Figure 5d, Table S2).

The hybrids 1, 2, 5, 7, 12, 15 to 25, 27, 37, 38, 45, 46, 48 to 59, 61, 71, and 72 have shown positive hybrid vigour for these four traits of seeds. Similarly, the hybrids 4, 8, 19, 26, 62, 63, 64, and 74 showed positive heterosis for three out of four traits.

DISCUSSION

Crossbreeding is a traditional approach to improve various plant species (Mukhtar and Mohamad, 2022), including horticultural crops, and remains one of the most successful strategies employed by breeders worldwide (Egorov, 2021). In this study, we have made significant strides in the development of new grape varieties with improved wine quality, yield, and values of bioactive compound through the crossing of Pinot Noir and Marselan. Our findings have potential implications for viticulture, as they contribute to the understanding of hybrid vigor in grape breeding and provide valuable insights for future wine industry and breeding programs.

In the past, China has developed grape varieties such as Beimei and Beihong by crossing Eurasian grape varieties with mountain grape, resulting in cultivars exhibiting drought resistance and high sugar content (Jiang *et al.,* 2010). Similarly, our hybrids have showed pronounced hybrid vigor in panicle and fruit yield over two years. Notably, one hybrid surpassed its parents in most yield-contributing traits, aligning with the phenomenon observed by Cao in F1 hybrids (Cao, 2014).

Wine quality is heavily influenced by grape fruit quality, juice composition, and the presence of various bioactive compounds (Rivero-Perez *et al.,* 2008), was a significant focus of our study. Our results paralleled those of Zhen (2007), where hybrid fruits showed higher titratable acid content than the superior parent, Pinot Noir (Zhen, 2007) (Su *et al.,* 2016). We further expanded our understanding of phenolic substances in wine grapes. Drawing comparisons to the work of Somkuwar *et al*. (2019), we evaluated the phenolic content across different parts of grape fruit. Our hybrid grapes exhibited varying hybrid vigor for these phenolics across the peel, flesh, and seeds. In previous studies Ban *et al.,* (2016) conducted a QTL analysis on a hybrid population, identifying a positive allele associated with increased berry weight, suggesting that fruit weight alterations may be quantitatively influenced. In line with previous studies, our research observed a broad range of hybrid vigor in panicle and fruit yield over two years, with one hybrid i.e., hybrid number 21 demonstrating superior hybrid vigor compared to its parents for most yield-contributing traits.

Similarly, Anthocyanin's influence on a wine's antioxidant properties was reinforced by Ju et al. (2021). Our observations aligned, with a notable difference being the presence of both red and green grape progenies after crossing two red grape varieties. This observation, in conjunction with findings from Liang et al. (2011), indicates a potential additive genetic model at play. Moreover, phenols, tannins, anthocyanins, and proanthocyanidins have shown positive impact wine quality (Kilmister *et al.,* 2014; Li and Sun, 2019; Rivero-Perez *et al.,* 2008) Our study suggests, the phenolic content in different parts of wine grape fruit varied and the total phenolic and tannin content in the seed hybrids were 2-3 times higher than that of their parents, indicating better heterosis for phenols, tannins, and anthocyanins. Similarly, proanthocyanidins are lauded for their antioxidant and anti-inflammatory properties (Unusan, 2020), our study found their content in the F1 generation to be comparatively lower. This negative heterosis defied our expectations and is an area ripe for future exploration.

Building upon our findings, practical grape breeding programs can focus on optimizing grape hybrids for enhanced wine quality and yield. A deeper genetic investigation into hybrid vigor and specific genetic factors influencing phenolic content is required. Such endeavors would undoubtedly benefit the viticulture industry. Furthermore, we speculate that our two-year results may aid in the selection of higher yielding grape hybrids for future breeding programs for Chinese wine industry. We used the female parent, Pinot Noir, to combine the desirable wine quality with the male parent, Marselan, which has a higher fruit yield but a greater peel-to-flesh ratio. The range of values obtained for their hybrids suggests that these two traits can be combined using the current parents. Our two-year study on 76 grape hybrids unearthed varying levels of hybrid vigor across different traits. Notably, hybrid number 25 displayed promising positive heterobeltiosis in specific traits. These findings pave the way for more focused genetic investigations and strategies to enhance grape production.

***Conclusion*:** Our two-year study on grape hybrid performance has unveiled critical insights into hybrid vigor, particularly in the context of fruit yield and wine-making quality. Certain hybrids, like hybrid number 25, have showcased positive heterobeltiosis for specific traits, marking them as promising candidates for the viticulture industry. By harnessing the potential of hybrids such as hybrid number 25 high-quality indigenous genotypes can make an impact in Chinese wine industry. In contrast, the negative heterobeltiosis of hybrids number 7 offers valuable insights into the complex genetic factors and their influence on phenotypic expressions, even if these hybrids are not immediately promising for direct commercial cultivation. Moving forward, there is a pressing need to dissect the genetic basis of observed heterosis patterns, which could unlock new avenues for combining desirable traits. Additionally, exploring the biochemical pathways influencing phenolic content and the correlation between these pathways and wine quality could yield invaluable insights for both breeders and wine-makers.

Abbreviations:

CV: Coefficient of Variation
Ta: Combined Transmissibility Rate
FY: Fruit yield
APW: Average Panicle Weight
ABW: Average Berry Weight
FSI: Fruit Shape Index
SFR Skin-to-Flesh Ratio
TSS: Total Soluble Solids
TA: Titratable Acids of juice
pH: pH of the juice
JY: Juice Yield
TPCF: Total Phenolic Content of Flesh
TCF: Tannin Content of Flesh
PCF: Proanthocyanidins Content of Flesh
TFCF: Total Flavonoids Content of Flesh
TPCP: Total Phenolic Content of Peel
TCP: Tannin Content of Peel
PAP: Proanthocyanidins Content of Peel
TACP: Total Anthocyanin Content of Peel
TFCP: Total Flavonoid Content of Peel
TPCS: Total Phenolic Content of Seed
TCS: Tannin Content of Seed
PCS: Proanthocyanidins Content of Seed
TFCS: Total Flavonoid Content of Seed

Conflict of Interest: The Authors declare that there is no conflict of interest.

***Author's contribution*:** ZL, ZD and QZ; conceives the idea. ZL conducted the research and collected the data with the help of BD MT, JH, JG. ZL, SS, US, ZD and QZ did the formal analysis and wrote the original draft; All the authors have read and approved the final version of the manuscript.

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