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Leaf Retention Influences Photosynthetic Activities, LAI and PAR in **Clone-2A Grapevine**

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ABSTRACT

he study was conducted during October, 2023 to March, 2024 at ICAR-National Research Centre for Grapes, Pune, ▲ Maharashtra, India to assess the leaf area requirement of Clone-2A grape variety. The experiment was laid out using Randomized Block Design (RBD) with variation in leaf on a fruit-bearing shoot such as 10,12,14,16 and >16 leaves above the bunch as five treatment replicated five times. The parameters measured were leaf area, leaf area index (LAI), photosynthetically active radiation (PAR), photosynthetic rate, stomatal conductance, chlorophyll content, average bunch weight, berry weight, yield vine⁻¹, total soluble solids (TSS) and acidity. Maximum bunch weight (450.60 g), 50-berry weight (182.60 g), TSS (18.25°B) and yield vine 1 (19.46 kg) were recorded in 14 leaves shoot above the bunch treatment. The highest photosynthetic activity such as assimilation rate (11.25 µmol CO₂ m⁻² s⁻¹) and transpiration rate (2.85 mmol H₂O m⁻² s⁻¹) and total chlorophyll content (35.28 mg ml⁻¹) was observed in the same treatment. Minimum bunch weight (380.10 g), 50-berry weight (155.22 g), TSS (16.60°B) and yield vine-1 (16.38 kg) were observed in >16 leaves above the bunch treatment. However, retaining 14 leaves above the bunch in a shoot with 2861.42 cm² leaf area shoot⁻¹, 85842.60 cm² leaf area vine⁻¹ and 1988.00 cm² leaf area bunch⁻¹ were found sufficient for quality grape production in Clone-2A grape variety spaced at 9×5 ft² distance.

KEYWORDS: Leaf retention, berry quality, photosynthetic rate, chlorophyll content

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

rape (Vitis vinifera L.) is one of the most widely Jcultivated fruit crops in the world, valued for their versatility and nutritional benefits. In 2024, grape production reached 3,896 thousand mt from an area of 176.91 thousand hectares (Anonymous, 2024a). During the fiscal year 2023-24, the country exported 343,982.34 metric tons, valued at 417.07 million USD (Anonymous, 2024b). The grapes are consumed fresh as table grapes, dried to produce raisins, or processed to produce wine, juice and other products. To produce quality grapes, leaves as a part of canopy play a crucial role. Leaves are considered powerhouses of photosynthesis, where chlorophyll absorbs light energy to convert carbon dioxide and water into carbohydrates. Young leaves generally start to produce excess photo assimilates, translocated afterwards to the other parts of the vine after they reach one-third of their full sizes, approximately 5 or 6 weeks after leaf unfolding (Candar et al., 2020). Depending on leaf age, regardless of whether they come from the lateral or main shoot, individual photosynthetic activities are effective in berry ripening and in sugar accumulation in grapes (Candar et al., 2020). This essential process not only stimulates plant growth but also supports the critical functions of evapotranspiration. Consequently, understanding leaf area requirement is crucial for physiological and agronomic studies related to plant development. The leaf surface area affects the yield and quality of grapevines. It is mainly influenced by variety, nutrition, canopy management practices like training and pruning and finally the age of plant (Burg et al., 2017). Leaf retention, the practice of maintaining leaves on the vine throughout the growing season plays a crucial role in the physiological processes of the grapevine (Somkuwar et al., 2014; 2024a; 2024b; Kakade et al., 2024). As leaf area index increases photosynthesis rate decreases (Somkuwar et al., 2019; 2024c). The LAI which measures the leaf area per unit ground area, is a critical parameter in understanding the canopy structure and its influence on photosynthesis, transpiration and ultimately, the yield and quality of the grape berries (Burg et al., 2017; Munitz et al., 2019; Somkuwar et al., 2024a; Kakade et al., 2024). Leaf removal also helps in organizing the vine source-sink balance (Moran et al., 2017; Somkuwar et al., 2024b). Managing the relationship between leaf area and fruit is a critical factor for achieving the desired fruit composition (Somkuwar et al., 2024b). Small differences in canopy microclimates, created by different leaf areas, can reduce the duration of grape maturity (Candar et al., 2020). Reducing leaf area can enhance nutrient intake for grape ripening and increase the photosynthetic activity of other leaves (Horak et al., 2021). Previous studies have determined the leaf area per unit weight of fruit to maximize sugar concentration, the most

used indicator of berry ripeness. Due to this phenomenon, it is important to point out the role of leaf removal practices into ripening processes. Several authors have reported that the minimum leaf area for adequate grape ripening per g of fruit is between 2.06 to 8.07 cm² g⁻¹ varying between cultivars (Somkuwar et al., 2024a; 2024b; 2024c and Kakade et al., 2024). However, different varieties have different leaf structures thereby creating a variation in the total leaf size. Considering the above, a study was conducted to investigate the effect of leaf retention above the bunch on leaf area, leaf area index, photosynthetic activity, yield and quality of Clone-2A grapes grafted on Dogridge rootstock under semi-arid conditions.

2. MATERIALS AND METHODS

The study was conducted during October, 2023 to March, ▲ 2024 at ICAR-National Research Centre for Grapes, Pune, Maharashtra, India. The experimental site was in mid-west Maharashtra at an altitude of 559 meters above mean sea level (18.32°N, 73.51°E). Clone-2A grapevines grafted onto Dogridge rootstocks were planted at a spacing of 9×5 ft² and trained to extended Y-trellis. About 0.5 cane/ ft² (24 canes vine⁻¹) were retained on each vine during foundation pruning. All the standard recommended cultural practices were followed to maintain the healthy vine during the period of study. After the fruit pruning, five treatments with variation in leaf number were evaluated as 10, 12, 14, 16 and >16 leaves above the bunch, each treatment was replicated five times. The experiment was conducted in a randomized block design (RBD). Leaf area index (LAI) and photosynthetically active radiation (PAR) were recorded using the LaiPen LP 110 device. Photosynthetic rate, stomatal conductance and transpiration rate were measured using an Infra-Red Gas Analyzer (IRGA model Li 6400, LI-COR Biosciences, NE, USA) on matured leaves (fifth to sixth from the tip) between 11 am and 12:30 pm. Leaf area was measured using the linear method (LBK method) with the formula: Leaf area (A)=L×B×K (0.810) and expressed in cm². Total leaf area shoot⁻¹, per vine and per bunch was calculated by multiplying the leaf area of the individual leaf by the number of leaves shoot⁻¹, shoots vine⁻¹ and dividing by the number of bunches vine⁻¹ respectively. The average bunch weight was derived from the mean weight of five randomly selected healthy bunches per replication, while the average weight of 50 berries was calculated and expressed in g. The number of berries bunch⁻¹ was averaged from five bunches per treatment. After maturity, grapes from five vines in each treatment were harvested and weighed to calculate the average yield vine-1 which was expressed in kilogs. Total soluble solid (TSS) was measured with a portable handheld refractometer (Erma Refractometer, Japan) at room temperature while total acidity (TA) was determined using OenoFoss (FTIR-based wine analyser) and expressed in g l⁻¹. Chlorophyll was extracted and estimated by Arnon's (1949) method.

2.1. Statistical analysis

The experiment was laid out using randomized block design (RBD) with five treatments and five replications. The Pearson correlation coefficient was used to determine the correlation between different growth and yield parameters. Data analysis was done using analysis of variance (ANOVA) performed as described by Panse and Sukhatme (1995).

3. RESULTS AND DISCUSSION

The effect of the number of leaves above the bunch on various parameters is presented in Table 1. The maximum leaf area leaf¹ was recorded in 10 leaves above the bunch (158.20) which was at par with 12 leaves above the bunch (155.60) treatment, while, the minimum values were recorded in more than 16 leaves above the bunch (135.30). With the leaves increased from 10 to >16 above the bunch, significant increase in the leaf area shoot⁻¹, vine and bunch was observed. The leaf area shoot⁻¹ increased

from 2373.00 cm² (10 leaves above the bunch) to 3382.50 cm² (>16 leaves above the bunch). Similarly, the leaf area vine⁻¹ also increased from 71190.00 cm² to 104857.50 cm² and the leaf area bunch⁻¹ from 1675.05 cm² to 2432.88 cm². The leaf area g⁻¹ of berry weight was increased as the leaf number increased above the bunch, which ranged from 3.98 cm² g⁻¹ to 6.40 cm² g⁻¹. Increased number of leaves above the bunch enhanced the overall leaf area, which can potentially boost the photosynthetic capacity (source) and resource distribution (sink) for grape development. Although, more leaves contribute to the overall vine foliage, there was a diminishing return in terms of total leaf area with each additional leaf. A more open canopy, resulting from increased leaf exposure to sunlight, leads to the synthesis of more photosynthetic assimilates as reported by Somkuwar et al. (2019). Candar et al. (2020) found similar results in Merlot grapes during their three-year study in Turkey, showing a desirable difference in leaf area vine-1 ranging from 1.92 to 6.54 m² vine⁻¹. Somkuwar et al. (2019) suggested that while a higher leaf count may contribute to increased photosynthetic activity, it does not necessarily result in a proportional increase in grapevine yield. Somkuwar et al.

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No. of leaves above a bunch	Leaf area leaf ⁻¹	Leaf area shoot ⁻¹ (cm ²)	Leaf area vine ⁻¹ (cm ²)	Leaf area bunch ⁻¹ (cm ²)	Leaf area g ⁻¹ berry wt (cm ² g ⁻¹)
10 leaves above bunch	158.20	2373.00	71190.00	1675.05	3.98
12 leaves above the bunch	155.60	2645.20	82001.20	1934.00	4.44
14 leaves above the bunch	150.60	2861.42	85842.60	1988.00	4.41
16 leaves above the bunch	145.20	3049.20	94525.20	2224.12	5.55
>16 leaves above the bunch	135.30	3382.50	104857.50	2432.88	6.40
SEm±	1.02	24.50	540.37	17.08	0.04
CD(p=0.05)	3.07	73.46	1620.02	51.22	0.13

(2024a) also reported that maintaining 12 leaves above the bunch, resulting in a leaf area of 63,820.80 cm² vine⁻¹, was optimal for achieving higher yield and improved quality in Crimson Seedless grapes. Similar results were observed in Manjari Kishmish and Nanasaheb Purple Seedless grape varieties (Somkuwar et al., 2024b; 2024c). Additionally, Kakade et al. (2024) also found comparable outcomes in the Manjari Medika grape variety.

The data recorded on the effect of different leaves above the bunch on yield and quality are presented in Table 2. The average bunch weight, berry diameter, 50-berry weight and yield vine⁻¹ ranged from 450.60 to 380.00 g, 18.20 to 17.10 mm, 182.60 to 155.22 g and 19.46 to 16.38 kg vine⁻¹ respectively. Maximum bunch weight, berry diameter, 50-berry weight and yield vine⁻¹ were recorded in 14 leaves above the bunch (450.60 g, 18.20 mm, 182.60 g and 19.46 kg respectively) followed by 12 leaves above

the bunch (135.12 g,17.85 mm, 174.10 g and 18.44 kg respectively). While, minimum was recorded in >16 leaves above the bunch (380.00 g,17.10 mm, 155.22 and 16.38 kg respectively). The number of bunches vines⁻¹ and the number of berries bunch-1 remained relatively stable and were non-significant across the treatments. The TSS was ranged from 18.25 to 16.60°Brix with highest in 14 leaves above the bunch (18.25°Brix), while, the lowest was recorded in >16 leaves above the bunch (16.60°Brix). Acidity was significantly varied across the leaf retention treatments with a minimum in >16 leaves above the bunch (5.00 g l^{-1}) followed by 12 leaves above the bunch (5.25 g l-1) and 14 leaves above the bunch (5.35 g l⁻¹), while, maximum acidity was recorded in 10 leaves above the bunch (5.50 g l⁻¹). The higher leaf numbers may reduce TSS, indicating potential dilution of sugar content in berries, which could affect sweetness and overall berry quality. Although, an increase

Table 2: Effect of leaves on bunch characters and yield in Clone-2A variety									
No. of leaves above a bunch	Average bunch weight (g)	Berry diameter (mm)	No of bunches vine ⁻¹	No of berries bunch ⁻¹	50-berry weight (g)	Yield vine ⁻¹ (kg)	TSS (°Brix)	Acidity (g l ⁻¹)	
10 leaves above the bunch	420.35	17.60	42.50	125.30	167.75	17.70	17.60	5.50	
12 leaves above the bunch	435.12	17.85	42.40	125.00	174.10	18.44	17.80	5.25	
14 leaves above the bunch	450.60	18.20	43.20	124.40	182.60	19.46	18.25	5.35	
16 leaves above the bunch	400.10	17.60	42.50	125.50	166.00	17.00	17.90	5.40	
>16 leaves above the bunch	380.00	17.10	43.10	125.40	155.22	16.38	16.60	5.00	
SEm±	3.26	0.12	NS	NS	1.31	0.14	0.17	0.03	
CD (p=0.05)	9.80	0.38	NS	NS	3.94	0.42	0.52	0.10	

in leaf number enhanced the total leaf area, it did not lead to improved bunch characteristics and yield. In fact, an excessive number of leaves might had a negative impact on both berry quality and yield. The result of the present study aligns with the findings of Candar et al. (2020) who reported the lowest yield (4.60 kg vine⁻¹) for the non-lateral shoot (NLS) and the highest (5.00 kg vine⁻¹) for half lateral shoot FLS (3-4 leaves). Korkutal et al. (2017) reported that complete removal of the lateral leaves increased the total acidity. Horák et al. (2021) also reported reduction of leaf area can favour nutrient intake, where the removal of 70% of leaf area led to the accumulation of lesser quantities of sugar in the grapes. Therefore, these grapes contained the lowest values of TSS. Similar to the TSS, TA increased with the leaf area. Cataldo et al. (2021) highlighted that reducing leaf number during berry growth could lead to changes in the growth curve of berries, affecting sugar accumulation and acid levels. Somkuwar et al. (2024a) observed that an increase in leaf area (10 to >16 leaves above a bunch) led to a reduction in total soluble solids (TSS) (range 18.20 to 17.20°Brix), while total acidity was increased (range 5.10 to 5.15 g l⁻¹) in Crimson Seedless grapes grafted on Dogridge rootstock. These findings also align with the results of Kakade et al. (2024) and further corroborate the studies by Somkuwar et al. (2024b, 2024c).

The effect of leaf number on leaf area index (LAI) and photosynthetically active radiation (PAR) is presented in Table 3. In the present study, LAI increased from 1.70 in 10 leaves above the bunch to 2.51 in >16 leaves above the bunch, this might due to widespread leaf coverage. However, PAR values decreased from 0.350 μ mol photon m^{-2} s $^{-1}$ to 0.155 μ mol photon m^{-2} s $^{-1}$. Higher leaf coverage increases shading and reduces light penetration to the leaves, potentially affecting photosynthesis. Similar results were also reported by Burg et al. (2017) in nine different grape varieties. Kang et al. (2022) reported LAI ranged from 0.8 to 2.4, 1.0 to 4.0 and 0.7 to 4.0 m²/m² in Cabernet Sauvignon, Chardonnay and Pinot Noir grapes respectively. Thoke et al.

Table 3: Effect of leaves on berry quality parameters in Clone-2A variety

No. of leaves above a bunch	LAI (m ² m ⁻²)	PAR (µmol photon m ⁻² S ⁻¹)
10 leaves above the bunch	1.70	0.350
12 leaves above the bunch	1.96	0.290
14 leaves above the bunch	2.05	0.200
16 leaves above the bunch	2.26	0.196
>16 leaves above the bunch	2.51	0.155
SEm±	0.017	0.0017
CD (p=0.05)	0.053	0.0052

(2024) conducted research on nine varieties and found the largest leaf area index (3.91 m²/m²) in Thompson Seedless while the lowest in Nanasaheb Purple Seedless (3.68 m²/ m²) and Crimson Seedless (3.87 m²/m²) after foundation pruning. Similar findings were reported by Somkuwar et al. (2024a, 2024b, 2024c) in Crimson Seedless, Manjari Kishmish and Nanasaheb Purple Seedless grape varieties under semi-arid condition. They observed that increasing the leaf area index (LAI), driven by a greater number of leaves above the bunch (ranging from 10 to more than 16), led to a decline in photosynthetically active radiation (PAR). Kakade et al. (2024) also reported a similar trend in the Manjari Medika variety, where an increase in leaf area above the bunch resulted in a higher LAI (ranging from 2.10 to 3.60), which consequently reduced PAR values (ranging from 0.30 to 0.95).

The effects of varying leaf density per shoot on photosynthetic parameters including assimilation rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate are summarized in Table 4. Assimilation rate was a key indicator of photosynthetic efficiency with the highest assimilation rate in 14 leaves above the bunch treatment (11.25 µmol CO_2 m⁻² s⁻¹) while, the lowest assimilation rate was observed in > 16 leaves above the bunch

Table 4: Effect	of leaves on	photosynthe	tic activities	in (Clone 2A	variety
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No. of leaves above bunch	Assimilation rate (μmol CO ₂ m ⁻² s ⁻¹)	Stomatal conductance (mmol CO ₂ m ⁻² s ⁻¹)	Intercellular CO ₂ (Ci) (ppm)	Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹)
10 leaves above the bunch	10.52	0.19	300.50	2.79
12 leaves above the bunch	11.00	0.20	310.20	2.81
14 leaves above the bunch	11.25	0.21	311.50	2.85
16 leaves above the bunch	9.35	0.18	289.60	2.68
>16 leaves above the bunch	9.10	0.18	288.10	2.61
SEm±	0.08	0.0016	2.24	0.02
CD(p=0.05)	0.25	0.0047	6.72	0.06

(9.10 μmol CO₂ m⁻² s⁻¹). However, it was observed that stomatal conductance increased slightly from 0.19 to 0.21 mmol CO₂ m⁻² s⁻¹ as the number of leaves increased from 10 to 14 leaves above the bunch. The highest intercellular CO₂ concentration was recorded in 14 leaves above the bunch treatment (311.50 ppm) while lowest in >16 leaves above the bunch (288.10 ppm). The transpiration rate ranged from 2.79 to 2.85 mmol H₂O m⁻² s⁻¹ for 10 leaves above the bunch to 14 leaves above the bunch, decreasing slightly to 2.61 mmol H₂O m⁻² s⁻¹ in >16 leaves above the bunch. The results of the present investigation were also in line with Somkuwar et al. (2014) who reported that canopy manipulation practices had no marked stimulating effect on stomatal conductance. The maximum rate of transpiration (3.05 µmol m⁻² s⁻¹) was recorded with shoot pinching at 10 leaves above the bunch in Tas-A-Ganesh grapes. Somkuwar et al. (2024d) reported that foliar biomass and leaf area were responsible for the alteration of gas exchange parameters in Manjari Naveen grapevine grafted into different rootstocks. The results of the present study confirm the findings of Kakade et al. (2024) in Manjari Medika and Somkuwar et al. (2024a; 2024b; 2024c) in Crimson Seedless, Manjari Kishmish and Nanasaheb purple Seedless grape varieties.

The chlorophyll a, b and total chlorophyll content ranged from 25.13 to 23.00, 10.15 to 7.85 and 35.28 to 30.85 mgml ¹ respectively (Table 5). However, maximum chlorophyll a, b and total chlorophyll were recorded in 14 leaves above the bunch (25.13, 10.15 and 35.28 mg ml⁻¹) treatment while, minimum in >16 leaves above the bunch (23.00, 7.85 and 30.85 mg ml⁻¹ respectively) treatment. Thus, the decrease in chlorophyll content with more leaves indicated reduced chlorophyll synthesis, or increased degradation, potentially affecting photosynthetic efficiency. Petrie et al. (2000) reported that leaf removal resulted in an increase in or retention of chlorophyll which also occurred for the full leaf removal crop treatment. Thoke et al. (2024) reported chlorophyll content ranged from 39.95 to 42.92 at 120 DAP and change in chlorophyll content due to the structure of the leaves, including size, thickness, shape and surface area

Table 5: Effect of leaves on chlorophyll content in leaves of Clone-2A variety (90 DAFP)

No. of leaves above bunch	Chlorophyll a (mg ml ⁻¹)	Chlorophyll b (mg ml ⁻¹)	Total chlorophyll (mg ml ⁻¹)
10 leaves above the bunch	23.50	9.13	32.63
12 leaves above the bunch	23.67	8.67	32.34
14 leaves above the bunch	25.13	10.15	35.28
16 leaves above the bunch	23.90	10.10	34.00
>16 leaves above the bunch	23.00	7.85	30.85
SEm±	0.17	0.06	0.24
CD (p=0.05)	0.53	0.20	0.74

which affects the distribution of chlorophyll while studying nine different grape varieties. Somkuwar et al. (2024a; 2024b; 2024c) reported in Crimson Seedless, Manjari Kishmish and Nanasaheb Purple Seedless grape varieties that beyond optimum leaf number above the bunch (10,12 and 14 leaves respectively), chlorophyll content per leaf was decreased as leaf area above the bunch increased.

The correlation analysis of various parameters related to leaf area index (LAI) varied significantly. LAI was positively correlated with leaf area vine⁻¹ (1.000) and leaf area bunch⁻¹ (0.999) with leaf area g⁻¹ of berry weight (0.966) indicating that as LAI increased, leaf area measurements also increased. Conversely, LAI was strongly negatively correlated with photosynthetically active radiation (PAR) (-0.932), this might due to higher LAI reduced light penetration in the canopy thereby disturbing the physiological balance of the grapevine. Negative correlations were also observed with total chlorophyll content (-0.287), average bunch weight (-0.684) and yield vine⁻¹ (-0.569) implying that increased

leaf area might reduce chlorophyll concentration and adversely affect fruit weight and yield. PAR also showed a moderate positive correlation with average bunch weight (0.430) and yield vine⁻¹ (0.287) indicating better light availability to enhance yield parameters. In the present study, total chlorophyll content was positively correlated with yield vine⁻¹ (0.694) and average bunch weight (0.668) indicating that chlorophyll content in the leaf was important

in vine productivity. Additionally, average bunch weight was positively correlated with yield vine⁻¹ (0.986), heavier bunches contributed significantly to the overall yield. This result was consistent with the findings of Kakade et al. (2024), who reported a strong positive correlation between average bunch weight (r=0.968), yield vine⁻¹ (r=0.943) and total chlorophyll content (r=0.972). Also, similar results had been reported by Somkuwar et al. (2024a, 2024b and 2024c).

Table 6: Correlation coefficients between different growth and yield parameters as influenced by number of leaves maintained above the bunch in Clone-2A

Parameters	Leaf area index (m² m-²)	PAR (µ mol photon m ⁻² S ⁻¹)	Leaf area vine ⁻¹ (cm ²)	Leaf area bunch ⁻¹ (cm ²)	Leaf area g ⁻¹ of berry wt. (cm ² g ⁻¹)	Total chlorophyll content (mg ml ⁻¹)	Av. bunch wt. (g)	Yield vine ⁻¹ (kg)
Leaf area index (m ² m ⁻²)	1							
PAR (μ mol photon m ⁻² S ⁻¹)	-0.932	1						
Leaf area vine ⁻¹ (cm ²)	1.000	-0.933	1					
Leaf area bunch ⁻¹ (cm ²)	0.999	-0.920	0.999	1				
Leaf area g^{-1} of berry wt. $(cm^2 g^{-1})$	0.966	-0.822	0.965	0.970	1			
Total chlorophyll content (mg ml ⁻¹)	-0.287	-0.048	-0.284	-0.295	-0.455	1		
Av. bunch wt. (g)	-0.684	0.430	-0.682	-0.697	-0.850	0.668	1	
Yield vine ⁻¹ (kg)	-0.569	0.287	-0.567	-0.589	-0.763	0.694	0.986	1

4. CONCLUSION

Clone-2A grapes revealed that retaining 14 leaves above the bunch was ideal for optimizing photosynthesis, resource allocation and berry quality. This treatment achieved the highest bunch weight, berry weight and yield vine-1 along with superior berry quality (TSS 18.25°B). In contrast, excessive leaf retention (>16 leaves) reduced efficiency and berry quality, emphasizing the critical role of precise canopy management in achieving sustainable and high-quality grape production under semi-arid conditions.

5. ACKNOWLEDGMENT

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