



ORIGINAL RESEARCH ARTICLE

Exploring key physiological attributes of grapevine cultivars under the influence of seasonal environmental variability

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ABSTRACT

Seasonal climatic variability is a key challenge in many grape-growing regions across the globe, affecting phenology, growth, physiological responses, and yield at harvest. Unfavourable climatic conditions impair the plant's physiological processes, such as chlorophyll accumulation, gas exchange and photosynthesis in grapevine leaves. It is critical to unlock the complex physiological behaviour of grapevine cultivars at key phenological stages and under varying environmental conditions. The present study was designed to evaluate the key physiological processes, such as gas exchange, chlorophyll contents and water use efficiency (WUE), of four table grape cultivars at key growth stages under varying environmental conditions of the Pothwar region, in a factorial experimental set up (Location × Year × Cultivar × Phenological stage). The physiological responses of the table grape cultivars were recorded at the 5-leaf stage, full bloom, berry set, veraison and harvest during two consecutive vintages (2019 and 2020) in two locations (Islamabad and Chakwal). The results show that the mean photosynthetic activity in colder Islamabad was 30.7 % higher than in Chakwal, and the transpiration rate and WUE were 10.4 % and 28.6 % higher. Similarly, 12 % higher photosynthetic activity, with 13 % more WUE, was observed in the colder vintage of 2020 compared to that of 2019. The vine physiological activity also varied among cultivars; for example, cv. Sugraone was found to have 12 % more chlorophyll and 30 % higher photosynthetic activity than cv. Kings Ruby. Similarly, higher photosynthetic activity and transpiration rates were recorded at the berry set stage, while WUE peaked near blooming. The biplot analysis for the first two principal components also showed cv. Sugraone to be a highly responsive and physiologically efficient cultivar. The findings of the present research will help to better assess the effect of seasonal variability on vine physiological performance and to identify genotypes with higher photosynthetic potential and WUE. It could also assist in devising vineyard management strategies to better adapt to varying environmental conditions.

KEYWORDS: Table grapes, photosynthetic responses, WUE, environmental variability, developmental stages

INTRODUCTION

Plant physiological processes (e.g., photosynthetic activity, chlorophyll synthesis, transpiration rate, and carbon dioxide uptake) vary with phenological stage and control seasonal plant growth responses (Williams and Jackson, 2007; Canton *et al.*, 2017). Vegetative phenology during the annual grapevine cycle is of high significance, because it is associated with the interception of light and its conversion into food reserves. Vines consume a reserve of carbohydrates from the previous season for bud burst or breaking dormancy, initiate shoot growth, and then start photosynthesis (Campos *et al.*, 2016). Grapevine physiological responses are temperature sensitive; projections show that high temperatures coupled with dry conditions will modify thermal environments and hence increase the evapotranspiration requirements of plants (Ficklin and Novick, 2017; Dusenge *et al.*, 2019). Chlorophyll accumulation in grapevines varies depending on its phenological stage: it increases rapidly with leaf expansion till flowering, and then stabilises and may gradually decrease near ripening (Canton *et al.*, 2017). Photosynthetic processes in plants are affected by temperatures above 35 °C; summer midday air temperatures in many grape growing regions can exceed 40 °C with deleterious effects on grapevine, particularly at berry ripening, thereby decreasing the yield and economic profitability of the vineyard (Salvucci and Crafts-Brandner, 2004; Liu *et al.*, 2012; Carvalho *et al.*, 2015). The photosynthetic efficiency of vine leaves is at its highest at 150-200 W/m light intensity, being necessary for good vegetative and reproductive growth; i.e., the source-sink relationship (de Assis *et al.*, 2004). It is critical to quantify grapevine genotypes according to water use efficiency (WUE) when selecting genotypes with adaptive traits under varying pedo-climatic conditions (Tomás *et al.*, 2014; Bota *et al.*, 2016; Tortosa *et al.*, 2019).

Grapevines adjust dynamically and acclimatise to environmental factors, such as temperature and light incidence. Shifts in Photosystem II (PSII) photochemistry have been observed with high summer temperatures and changes in sink demands (Greer, 2019). The physiological responses of grapevine vary depending on the key growth stage; for example, peak photosynthetic activities have been recorded near the berry set stage (Somkuwar *et al.*, 2014; Greer *et al.*, 2020). In addition to berry set, veraison to harvest is a period of high physiological activity that affects berry growth, quality and yield (Rogiers, *et al.*, 2017). Moreover, vintage climatic variations highly influence vine physiology and berry ripening (Rienth *et al.*, 2020). Extreme weather conditions during the active growth season affect vine growth, berry development and physiological processes; for example, the inhibition of photosynthesis due to the impairment of electron transport activity, resulting in a low economic return due to low fruit set and low berry quality at ripening (Murakami *et al.*, 2000; Greer, 2012).

The Intergovernmental Panel on Climate Change (IPCC) has stated that the last four decades have been successively the warmest since 1850. Recent projections indicate an increase in terrestrial temperature of 1.59 °C over the past two decades of this century compared to the pre-industrial period (1850-1900). Climate change projections indicate that global mean temperature could increase by 1.7 °C according to the RCP2.6 scenario and by 4.8 °C according to the RCP8.5 scenario by the end of this century, coupled with an increase in atmospheric CO₂ levels (IPCC, 2013; IPCC, 2021). Climate change has also affected global viticultural regions, and an increase of 0.5 °C in the growing season temperature (GST) has been observed in 76 % of grape growing regions for the period 1989 to 2018 compared to the preceding 30-year period (1959 to 1988).

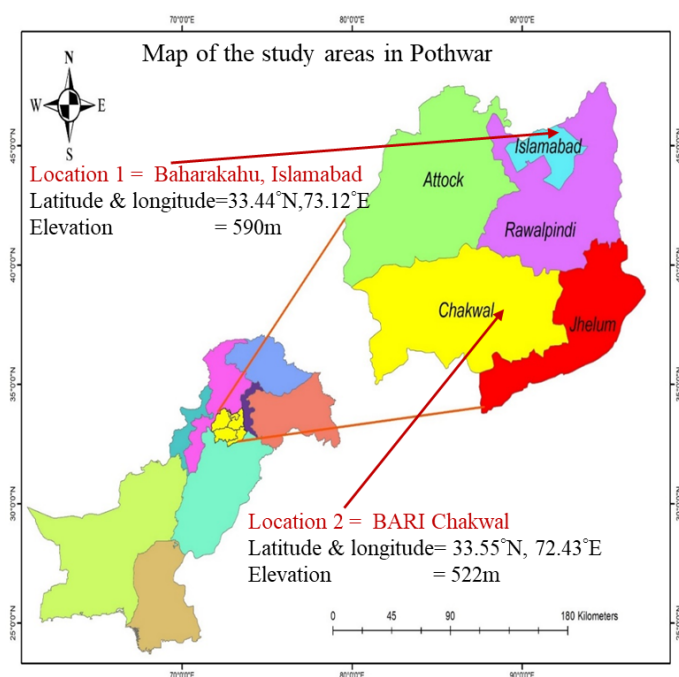


FIGURE 1. Map of the Pothwar region in Pakistan showing the two study sites.

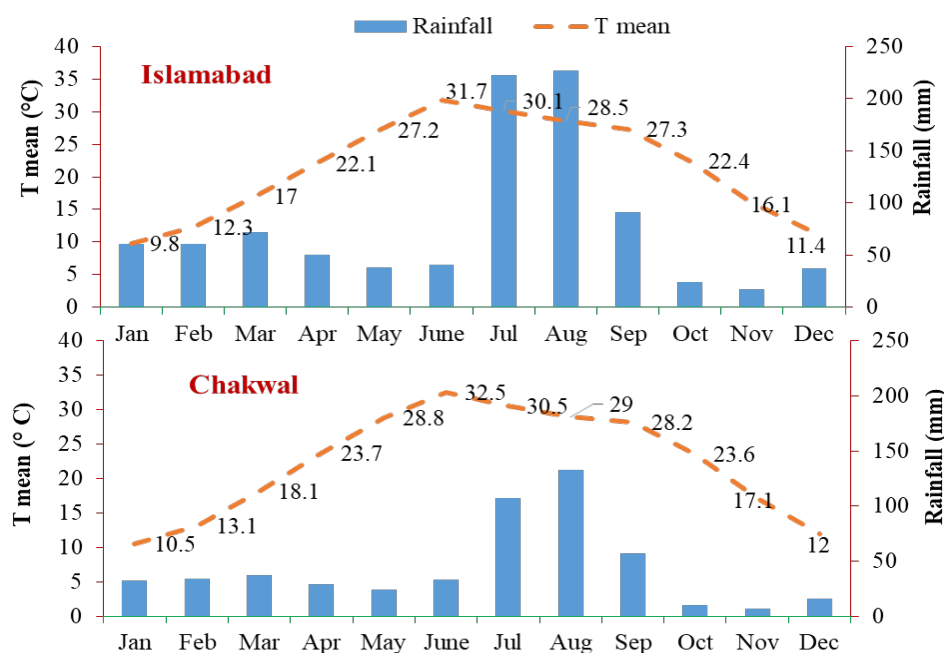


FIGURE 2. Historical (1980-2010) mean monthly temperature at the study sites.

A similar increase of more than 1 °C has been recorded in 46 % of the traditional viticultural regions (Puga *et al.*, 2022). These warming trends have altered grapevine phenology, physiology and quality in existing viticultural regions (Arias *et al.*, 2022; Rafique *et al.*, 2021; Rafique *et al.*, 2023b; Shah *et al.*, 2021), which may cause a shift in present day grape producing regions. Extreme heat coupled with water stress, particularly during ripening, will abruptly reduce grapevine metabolism, resulting in higher sugar levels with low acidity and less yield (De Orduna, 2010; Fraga *et al.*, 2018); this is a potential threat to the sustainability of global viticultural systems.

Grapevine physiology is negatively affected by elevated temperatures in warm climates and by lower temperatures in temperate climates (Koörner, 2007; Greer and Weedon 2013; Rafique *et al.*, 2023a), thus indicating that grapevines are highly sensitive to environmental variables; for instance, the amount of photosynthate transferred from the source (the leaf) is dependent on the leaf carbon balance, which is affected by the leaf's metabolic activity and rate of photosynthesis (Taiz and Zeiger, 2006; Keller, 2020). An increase in temperature will initially augment stomatal aperture with positive effects on leaf photosynthesis; however, very high temperatures cause stomatal closure, resulting in lower stomata conductance and photosynthetic activity (Lammertsma *et al.*, 2011; Keller, 2020; Arrizabalaga-Arriazu *et al.*, 2020). Climatic variability has a negative effect on the physiological activities of vines such as, photosynthetic activity, transpiration and stomatal conductance which lower water use efficiency (WUE), resulting in higher irrigation demands (Rafique *et al.*, 2023a). It is feared that due to this variability, the traditional grape growing area will decrease by about 20 % (Sgubin *et al.*, 2023). Hence, it is necessary to explore the possibility of establishing new viticultural zones to meet the future needs of this high value fruit crop.

Recently, the Pothwar plateau of Pakistan has emerged as a potential new viticultural zone for table grape cultivars in southern Asia (Rafique *et al.*, 2021; Anjum *et al.*, 2020; Anjum *et al.*, 2022). It has distinct climatic conditions: from humid subtropical to warm semi-arid, with air temperature varying between below 0 °C (winter) to above 40 °C (summer), along with fluctuating rainfall patterns (Sarfaraz *et al.*, 2014). A few recent studies have shown accelerated phenology of the table grape cultivar in this region, thus a shortening of the growth season, due to rapid GDD accumulation (Rafique *et al.*, 2020; Rafique *et al.*, 2023b). However, there is still a significant lack of knowledge regarding the physiology of different grapevine cultivars grown under the climatic conditions of the Pothwar region. Moreover, the currently grown commercial cultivars may not remain suitable with future warming. Therefore, monitoring the physiology of grapevines under varying environmental conditions could help to maintain vine balance, as well as high photosynthesis and WUE for better yield. The goal of this study was to explore the grapevine physiological responses at key developmental stages to the environmental conditions of the Pothwar region in southern Asia.

MATERIALS AND METHODS

1. Site and plant material

Two main grape growing areas in the Pothwar region about 120 km apart were selected for the physiological assessment: Islamabad (33.44° N, 73.12° E) and Chakwal (32.55° N, 72.43° E) (Figure 1). The Chakwal district has an elevation of 522 m and the Islamabad district is located 590 m above sea level, with high seasonal climatic variability at both locations. The experiment was carried out in a Randomised Complete Block Design (RCBD) with four factors: locations

(Islamabad and Chakwal), vintages (2019 and 2020), cultivars (King's Ruby, Perlette, NARC Black and Sugraone) and phenological stages (5 leaf stage, blooming, berry set, veraison and maturity stage). The germplasm for NARC Black cultivar was obtained from the National Agriculture Research Center (NARC), Islamabad Pakistan, and had been developed through selection of local germplasm. The four selected table grape cultivars (*Vitis vinifera*), King's Ruby (red), NARC Black (black), Perlette and Sugraone (yellowish-green), are considered as suitable and high potential cultivars for this region based on their phenology as discussed by Rafique *et al.* (2023b).

2. Vine selection and vineyard management

In both locations, healthy 5–6-year-old fruit-bearing vines of uniform vigour, trellis system and productivity potential were selected during the growing season of 2018 near fruit ripening stage. A total of nine vines (3 vines per replication) of each cultivar were randomly selected at both locations for the measurement of physiological responses. Both vineyards were managed by applying standard regional viticultural practices. The vines were trained on a bilateral, vertical positioning system (VPS), with a trunk height up to 1.7 m and plant and row spacing of 2.4×3.0 m. Vines of cvs. King's Ruby, Perlette and NARC Black were spur-pruned to 2 buds in winter during the last week of January, while cv. Sugraone was cane-pruned to 8–10 buds in both vineyards for all cultivars. Two canes were retained on each side of the vine with 8–10 buds each, thus a total of 32–40 buds were retained on each vine. Irrigation was applied in furrows when required to avoid water stress. A soil moisture meter (Aquamter M-200) was used for maintaining the soil conditions at near field capacity in the vineyards.

The total irrigation applied with this arrangement was 420 mm at Chakwal and 380 mm at Islamabad for the 2019 vintage, whereas for the 2020 vintage it was 240 mm at Chakwal and 200 mm at Islamabad. Fertilisers were applied in the following quantities to each vine in both locations: 250 g of N, 250 g of P_2O_5 , and 200 g of K_2O , as well as 20 kg of Farmyard manure (FYM).

3. Soil profile of the experimental vineyards

Soil analysis was performed for both locations at the start of experimentation in December 2018 before adding any fertiliser. The soil texture at Islamabad was 'silty clay loam' with 0.69 % organic carbon (OC), 9 mg/kg nitrate-nitrogen (NO_3-N), 1.40 mega gram per cubic meter (Mg/m) bulk density (BD) and a pH of 7.86. The soil at Chakwal was 'loam' with 0.60 % OC, 7 mg/kg NO_3-N , 1.42 Mg/m bulk density (BD) and a pH of 8.19. The water holding capacity of the soil at Islamabad was 39.7 %, while at Chakwal it was 26.2 %.

4. Climatic variability of the locations

The historical climatic data (1980–2010) collected from the weather stations of the Pakistan Meteorological Department (PMD) for the Chakwal and Islamabad districts showed variable temperature and rainfall trends in both locations (Figure 2). Semi-arid climatic conditions with an average annual temperature of 22.3 °C and annual rainfall of 519 mm prevail in Chakwal, while Islamabad is subject to sub-humid climatic conditions with an average annual temperature of 21.3 °C and annual rainfall of 987 mm (<http://climate-data.org>). The mean monthly temperature differed between the two locations by up to 1.6 °C, Islamabad being colder and more humid than Chakwal.

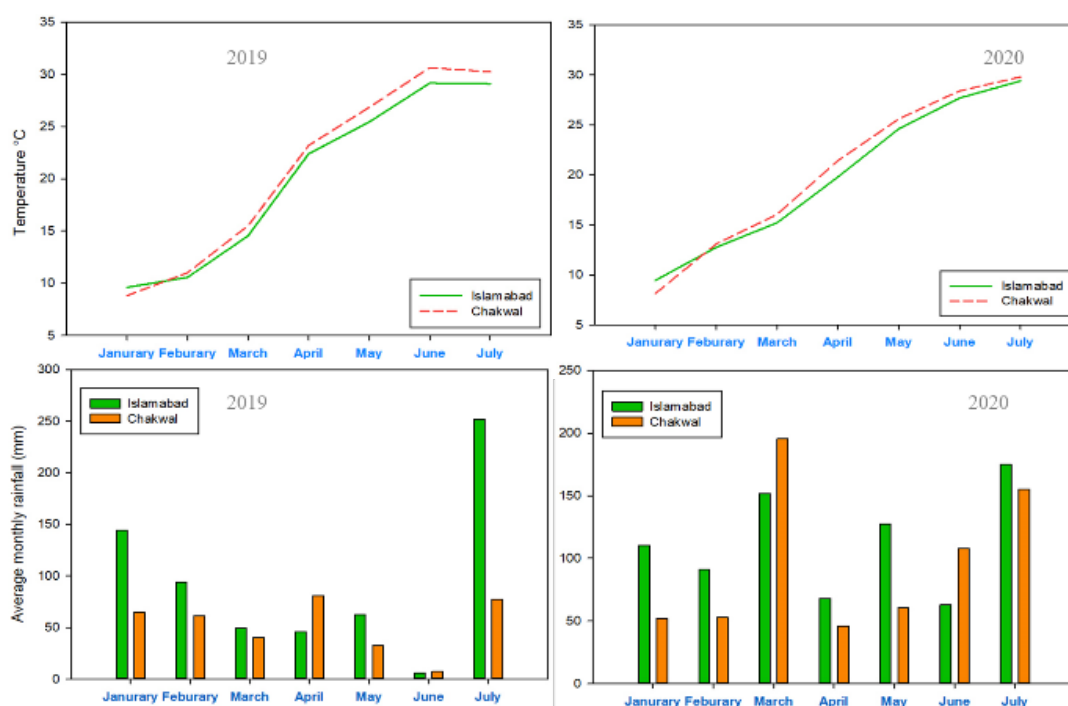


FIGURE 3. Study site average monthly rainfall and mean temperature.

TABLE 1. Onset dates of key phenological stages in different grapevine cultivars.

Cultivar	Phenological Stage	2019		2020	
		Islamabad	Chakwal	Islamabad	Chakwal
Kings Ruby	5-Leaf Stage	02 Apr	29 Mar	22 Mar	18 Mar
	Full Bloom	30 Apr	24 Apr	27 Apr	22 Apr
	Berry Set	6 May	28 Apr	3 May	26 Apr
	Veraison	15 Jun	8 Jun	17 Jun	10 Jun
	Harvest	06 Jul	27 Jun	8 Jul	30 Jun
Perlette	5-Leaf Stage	31 Mar	26 Mar	17 Mar	13 Mar
	Full Bloom	26 Apr	20 Apr	22 Apr	18 Apr
	Berry Set	30 Apr	24 Apr	28 Apr	23 Apr
	Veraison	9 Jun	31 May	9 Jun	3 Jun
	Harvest	30 Jun	20 Jun	1 Jul	23 Jun
NARC Black	5-Leaf Stage	05 Apr	01 Apr	27 Mar	22 Mar
	Full Bloom	21 Apr	15 Apr	17 Apr	14 Apr
	Berry Set	27 Apr	20 Apr	24 Apr	19 Apr
	Veraison	24 Jun	14 Jun	27 Jun	16 Jun
	Harvest	18 Jul	08 Jul	20 Jul	11 Jul
Sugraone	5-Leaf Stage	31 Mar	26 Mar	19 Mar	14 Mar
	Full Bloom	30 Apr	24 Apr	29 Apr	23 Apr
	Berry Set	5 May	27 Apr	4 May	27 Apr
	Veraison	12 Jun	4 Jun	14 Jun	6 Jun
	Harvest	11 Jul	2 Jul	12 Jul	6 Jul

Both districts are subject to strong summer monsoons, which produce about 70 % of annual rains.

Growing season weather data (January to July) for the two locations were collected from a NARC (National Agriculture Research Centre) weather station (33.40°, 73.13°) located 12.5 km from the Islamabad experimental vineyard, and from a SAWCRI (Soil and Water Conservation Research Institute) weather station (32.55°, 72.43°) located about 1 km from the Chakwal site. Automatic data loggers (Benetech GM 1365, data range -30 °C to 80 °C) were also installed at both locations for on-site temperature measurements. Data loggers (one for each site) were placed inside a Stevenson screen as per standards of the World Meteorological Organization (WMO) for air temperature measurement. Air temperature was recorded from an on-site temperature recording facility developed by our team, whereas rainfall data were collected from weather stations located in the respective districts. This setup allowed us to obtain more precise on-site temperature measurements.

5. Recording of physiological data at key phenological stages

The physiological responses of the table grape cultivars, King's Ruby, Perlette, NARC Black and Sugraone, were measured at five key phenological stages in 2019 and 2020: 5-leaf stage, blooming, berry set, veraison and maturity.

The physiological data were recorded when 50 % of the sample vines had attained the phenological growth stage under consideration as shown in Table 1.

The measurements were taken at the 5th distal and fully expanded leaf near the 4th to 5th node position of the reproductive shoot as described by Greer (2020). Each time, the data were recorded from leaves fully exposed to sunlight at the same nodal position of the branch. The data were recorded from a total of eighteen randomly selected healthy leaves at specific nodal positions for each cultivar, as previously described. The same leaves were used each time for chlorophyll and other physiological measurements. All the physiological measurements were recorded on a sunny day between 10:00 and 12:00, as described by Greer and Weedon (2013). Photosynthetic activity ($\mu\text{mol}/\text{m}^2/\text{s}$), transpiration rate ($\text{mmol}/\text{m}^2/\text{s}$), stomatal or leaf conductance ($\text{mmol}/\text{m}^2/\text{s}$), sub-Stomatal or Intercellular CO_2 concentration (Ci) $\mu\text{mol}/\text{mol}$ and intrinsic Water Use Efficiency (WUE) were recorded using portable photosynthesis system-3 (CIRAS-3) and applying the method described by Long and Bernacchi (2003). The leaf chlorophyll content index (CCI) was also determined at key phenological stages (5-leaf stage, blooming, berry set, veraison and maturity stage) at both locations using a portable chlorophyll content meter (CCM-200 plus).

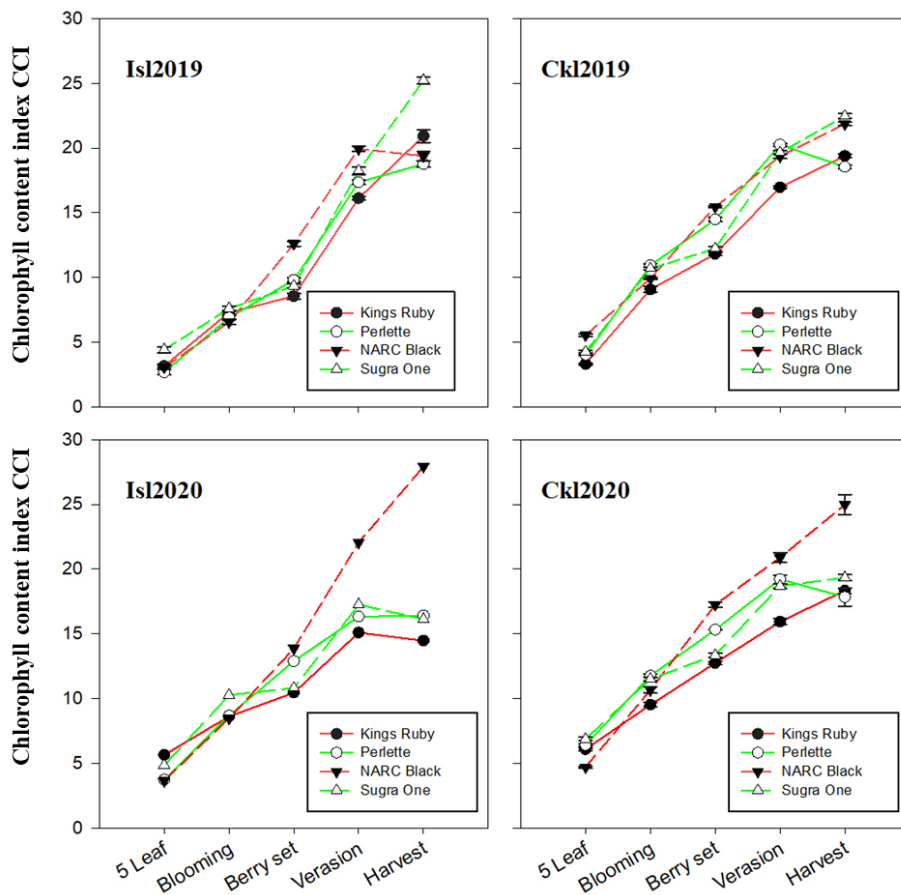


FIGURE 4. Chlorophyll index (CCI) of grapevine cultivars at key growth stages.

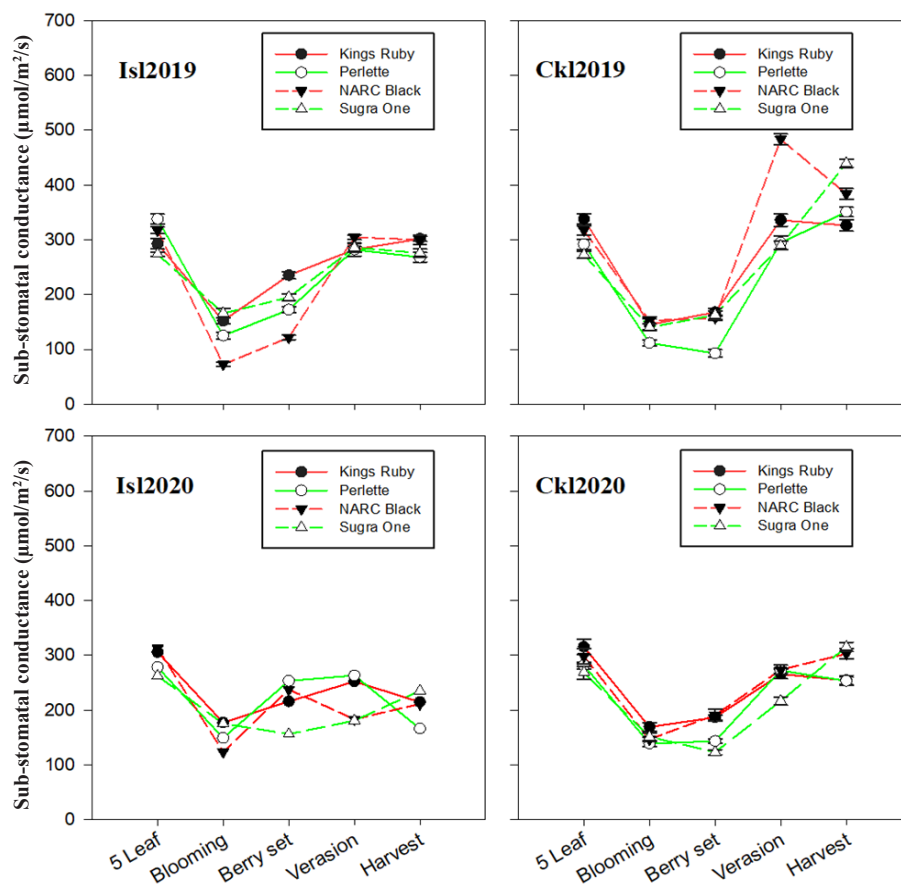


FIGURE 5. Evolution of sub stomatal conductance in grapevine cultivars over key growth stages.

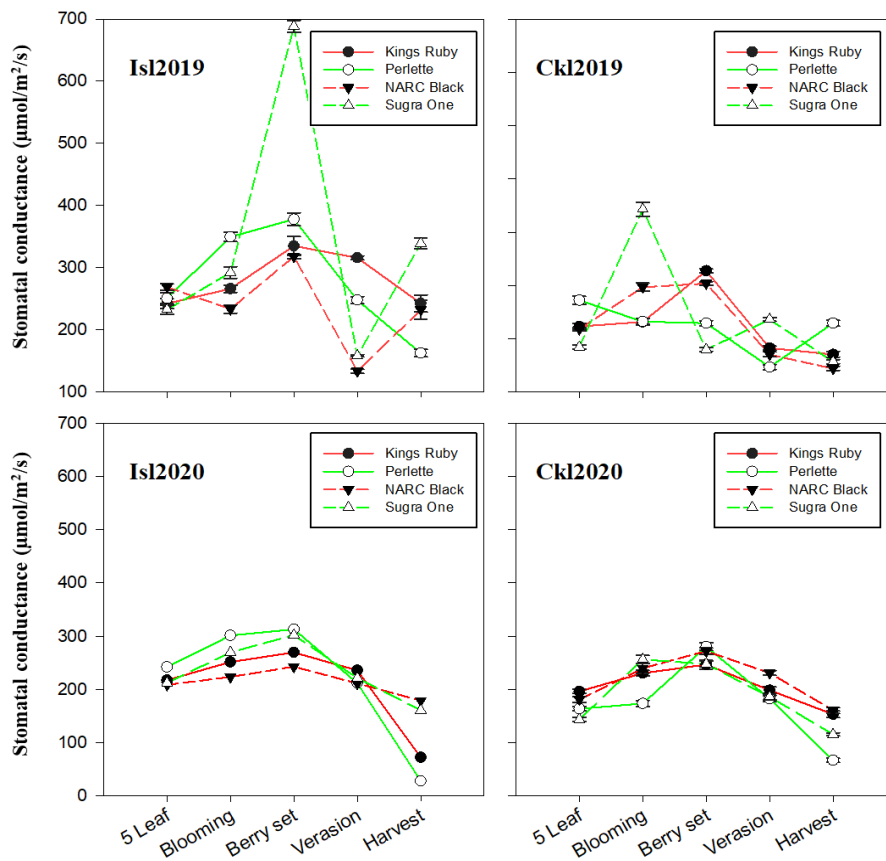


FIGURE 6. Stomatal conductance of grape cultivars at key growth stages.

6. Data analysis

The results were statistically analysed using Analysis of variance (ANOVA) techniques and the means were compared using Tukey HSD test at a 5 % probability level using software Statistcix 8.1 (Gomez and Gomez, 1984). Sigma Plot graphical software and MS Excel were used for data visualisation. For the differentiation and better visualisation of the variability of distinct environments (between locations and years), cultivars and physiological attributes, a principal component analysis (PCA) was also performed using XLSTAT (<https://www.xlstat.com/en/>).

RESULTS

1. Variable environmental conditions in the growing season

Varying environmental patterns were observed for the 2019 and 2020 vintages at both locations, with the Islamabad district having relatively lower temperatures and receiving more rainfall (Figure 3). The mean active growing season temperature (GST) from March to July (bud burst to harvest period) was 24.2 °C (Islamabad) and 25.1 °C (Chakwal) for the 2019 vintage, whereas for the 2020 vintage, it was 23.0 °C (Islamabad) and 24.2 °C (Chakwal). For the 2020 vintage, GST was 1.2 °C and 0.9 °C lower than in 2019 at Islamabad and Chakwal respectively. Similarly, compared to Islamabad, the growing season minimum temperature (T min) was higher at Chakwal by up to 1.6 °C, while the maximum temperature (T max) was higher by up to 2.58 °C. The prevailing environmental conditions resulted in 2020

being a colder vintage than 2019. Moreover, relatively higher seasonal rainfall was observed at Islamabad district (up to 41 mm) compared to Chakwal.

2. Leaf chlorophyll content

The results show the total chlorophyll content index (CCI) to be highly variable; it differed significantly between the two locations with mean values being higher at Chakwal (13.5) than at Islamabad (12.4). Similarly, CCI differed between vintages, being higher for the 2020 vintage (13.2) than for the 2019 vintage (12.7). When comparing the table grape cultivars, the mean CCI value was significantly high in cv. NARC Black at both locations, while that of cv. Kings Ruby was significantly low (11.59) (Table 2). Leaf chlorophyll increased linearly through the phenological stages (i.e., 5-leaf stage, full bloom, berry set, veraison and harvest) with values of 4.1, 9.1, 12.2, 18.4 and 20.8 respectively (Table 2 and Figure 4). The source of variation (SOV) for all the factors (i.e., phenological stage (PS), location (L), cultivar (C) and year (Y)) was highly significant. Similarly, all the two-way (PS*L, PS*C, PS*Y, L*C, L*Y, C*Y), three-way (PS*L*C, PS*L*Y, PS*C*Y, L*C*Y) and four-way (PS*L*C*Y) interactions varied significantly (Table 3).

3. Sub-stomatal conductance (Ci)

Inter leaf CO₂ concentration or sub-stomatal conductance (Ci) is highly variable and it varied significantly depending on phenological stage, location, vintage and cultivar (Table 2 and Figure 5). Higher Ci was recorded at Chakwal (243.9 µmol/m²/s) than at Islamabad (227.9 µmol/m²/s). The mean Ci of table grape cultivars differed significantly

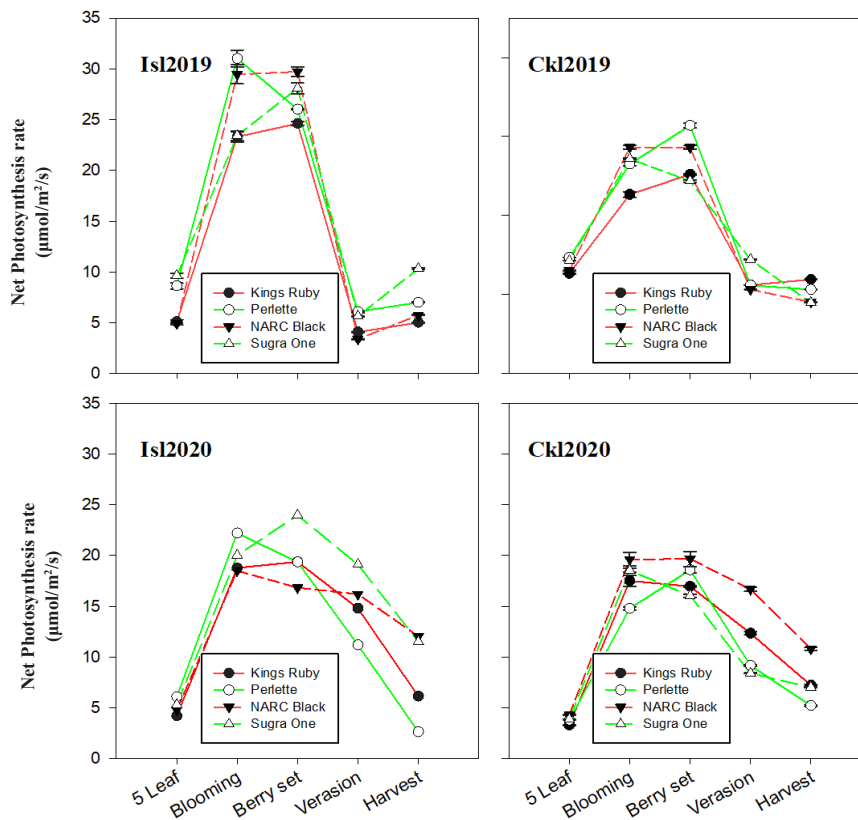


FIGURE 7. Evolution of net photosynthesis in grapevine cultivars over key growth stages.

TABLE 2. Grape physiological responses for vintages, locations and growth stages.

		CCI	Ci	gs	A	E	WUE
Years	2019	12.71B	248.71A	206.78A	11.219B	4.939A	2.099B
	2020	13.19A	223.12B	207.57A	12.673A	4.972A	2.446A
	LSD	0.0665	4.5703	3.5221	0.1643	0.0473	0.0360
Locations	Islamabad	12.38B	227.89B	251.62A	14.109A	5.226A	2.653A
	Chakwal	13.52A	243.93A	162.73 B	9.784B	4.686B	1.892B
	LSD	0.066	4.5703	3.5221	0.1643	0.0473	0.0360
Cultivars	Kings Ruby	11.59D	246.65A	205.57B	10.825C	4.723C	2.102C
	Perlette	13.00 C	223.12B	198.18C	11.815B	4.785 C	2.369A
	NARC Black	14.08 A	244.60A	198.53C	12.636A	5.034B	2.344A
	Sugraone	13.14 B	229.28B	226.40 A	12.509A	5.280A	2.276B
	LSD	0.123	8.470	6.527	0.304	0.088	0.067
Phenological stages	5 Leaf	4.10E	297.69A	191.21C	4.919 E	4.045E	1.212E
	Bloom	9.11D	143.60E	243.27B	20.136B	5.376B	3.747A
	Berry Set	12.26C	175.77D	283.40A	20.554A	6.245A	3.355B
	Version	18.45B	275.06C	179.48 D	8.412 C	4.472D	1.742C
	Harvest	20.84A	287.44B	138.50 E	5.710D	4.640 C	1.309D
	LSD	0.1464	10.055	7.7485	0.3614	0.1041	0.0792

CCI = Chlorophyll content index, Ci = sub-stomatal conductance ($\mu\text{mol}/\text{m}^2/\text{s}$), gs = stomatal conductance ($\mu\text{mol}/\text{m}^2/\text{s}$), A = photosynthetic activity ($\mu\text{mol}/\text{m}^2/\text{s}$), E = transpiration rate ($\text{mmol}/\text{m}^2/\text{s}$), WUE = water use efficiency as A/E ratio ($\mu\text{mol}/\text{mmol}$).

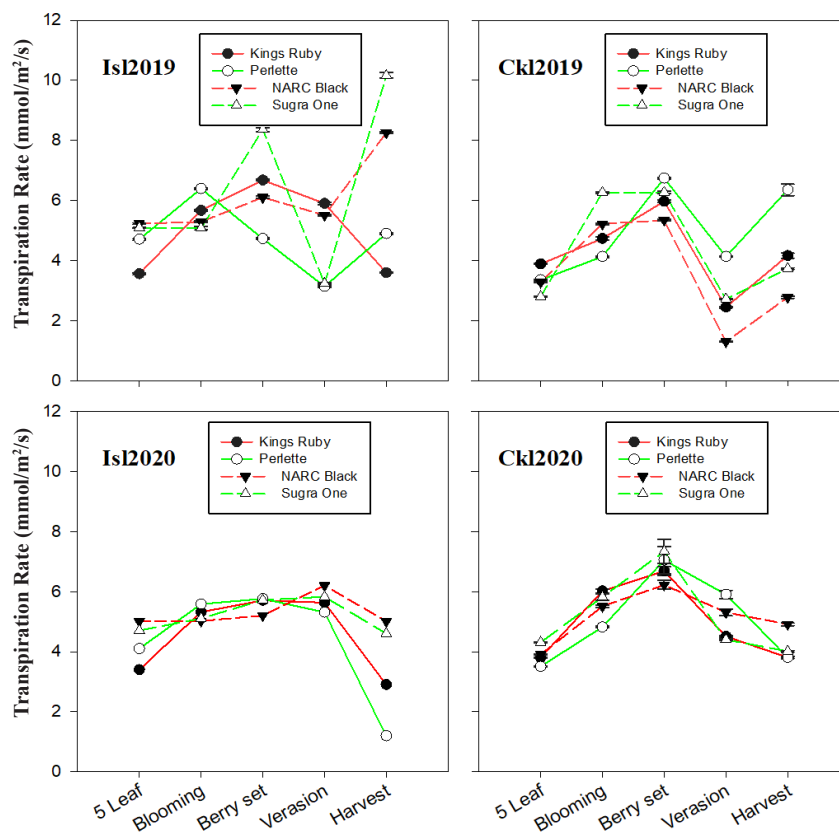


FIGURE 8. Evolution of transpiration rate in grapevine cultivars at key growth stages.

between the two vintages, with a higher C_i being recorded in the 2019 vintage ($248.5 \mu\text{mol}/\text{m}^2/\text{s}$) than in the 2020 vintage ($223.1 \mu\text{mol}/\text{m}^2/\text{s}$). Differences were also found between the C_i values at grape cultivar level: sub-stomatal conductance was higher in cvs. Kings Ruby ($246.65 \mu\text{mol}/\text{m}^2/\text{s}$) and NARC Black ($244.60 \mu\text{mol}/\text{m}^2/\text{s}$) than in cvs. Perlette ($223.12 \mu\text{mol}/\text{m}^2/\text{s}$) and Sugraone ($229.28 \mu\text{mol}/\text{m}^2/\text{s}$). Elevated levels of C_i were recorded at the early growth (i.e., 5-leaf ($297.69 \mu\text{mol}/\text{m}^2/\text{s}$)) and late developmental (i.e., veraison ($275.06 \mu\text{mol}/\text{m}^2/\text{s}$)) stages, and at harvest ($287.44 \mu\text{mol}/\text{m}^2/\text{s}$) in comparison with the bloom and berry set stages (Table 2). Source of variation (SOV) for all the factors and their interactions were significant (0.05), whereas the three-way interaction $\text{PS}^*\text{L}^*\text{Y}$ was non-significant (Table 3).

4. Stomatal conductance (g_s)

Stomatal conductance (g_s) also differed significantly at the key developmental stages between cultivars and between locations, while the yearly variations were non-significant (Figure 6 and Table 2). Higher stomatal conductance (g_s) was recorded at Islamabad ($251.62 \mu\text{mol}/\text{m}^2/\text{s}$) than at Chakwal ($162.73 \mu\text{mol}/\text{m}^2/\text{s}$). When comparing the cultivars, higher g_s values were obtained for Sugraone ($226.40 \mu\text{mol}/\text{m}^2/\text{s}$) and Kings Ruby ($205.57 \mu\text{mol}/\text{m}^2/\text{s}$) than for Perlette ($198.18 \mu\text{mol}/\text{m}^2/\text{s}$) and NARC Black ($198.53 \mu\text{mol}/\text{m}^2/\text{s}$). The highest g_s activity was observed at berry set ($283.40 \mu\text{mol}/\text{m}^2/\text{s}$) and the blooming stage ($243.27 \mu\text{mol}/\text{m}^2/\text{s}$), while the lower values of $191.21 \mu\text{mol}/\text{m}^2/\text{s}$, $179.48 \mu\text{mol}/\text{m}^2/\text{s}$, $138.50 \mu\text{mol}/\text{m}^2/\text{s}$ were observed at the 5-leaf, veraison and

harvest stages respectively. The source of variation (SOV) for the factors PS, L and C was highly significant; however, it was non-significant for the years. Similarly, all the two-way (PS^*L , PS^*C , PS^*Y , L^*C , L^*Y , C^*Y), three-way ($\text{PS}^*\text{L}^*\text{C}$, $\text{PS}^*\text{L}^*\text{Y}$, $\text{PS}^*\text{C}^*\text{Y}$, $\text{L}^*\text{C}^*\text{Y}$) and four-way ($\text{PS}^*\text{L}^*\text{C}^*\text{Y}$) interactions varied significantly (Table 3).

5. Photosynthetic activity (A)

The photosynthetic activity (A) of the grapevine cultivars varied significantly depending on phenological stage under varying environmental conditions (Figure 7 and Table 2). Higher 'A' values were observed at Islamabad ($14.1 \mu\text{mol}/\text{m}^2/\text{s}$) than at Chakwal ($9.8 \mu\text{mol}/\text{m}^2/\text{s}$), and in terms of vintages, photosynthetic activity was higher during the 2020 vintage ($12.7 \mu\text{mol}/\text{m}^2/\text{s}$) than during the 2019 vintage ($11.2 \mu\text{mol}/\text{m}^2/\text{s}$). Regarding the cultivars, higher mean 'A' activity was recorded in cvs. NARC Black and Sugraone than in cvs. Kings Ruby and Perlette. Grapevine photosynthetic activity rapidly increased with phenological progression from 5-leaf to berry set stage (i.e., from 4.9 to $20.6 \mu\text{mol}/\text{m}^2/\text{s}$), and after attaining peak values it rapidly decreased ($5.7 \mu\text{mol}/\text{m}^2/\text{s}$) at maturity. Source of variation (SOV) for all factors (PS, L, C and Y) and their interactive effects varied significantly (Table 3).

6. Transpiration rate (E)

The mean transpiration rate (E) varied significantly (0.05) among the cultivars and the phenological stages at both locations; however, it was not affected by yearly variations, as the responses in both vintages varied non-significantly (Figure 8 and Table 2).

TABLE 3. Output of analysis of variance indicating significance of key physiological attributes for growth stages, locations, cultivars, years, and their interactive effects.

Source	A	C _i	Cl	E	G	(WUE)
PS	sig	sig	sig	sig	sig	sig
L	sig	sig	sig	sig	sig	sig
C	sig	sig	sig	sig	sig	sig
Y	sig	sig	sig	NS	NS	sig
PSxL	sig	sig	sig	sig	sig	sig
PSxC	sig	sig	sig	sig	sig	sig
PSxY	sig	sig	sig	sig	sig	sig
LxC	sig	sig	sig	sig	sig	sig
LxY	sig	sig	sig	sig	sig	sig
CxY	sig	sig	sig	sig	sig	sig
PSxLxC	sig	sig	sig	sig	sig	sig
PSxLxY	sig	NS	sig	sig	sig	sig
PSxCxY	sig	sig	sig	sig	sig	sig
LxCxY	sig	sig	sig	sig	sig	sig
PSxLxCxY	sig	sig	sig	sig	sig	sig

Y = Years, C = Cultivar, L = Locations, PS = Phenological stage.

CCI = Chlorophyll content index, C_i = sub-stomatal conductance, g_s = stomatal conductance, A = photosynthetic activity, E = transpiration rate, WUE = water use efficiency.

sig = significant (0.05), NS = non-significant.

A higher transpiration rate (E) was recorded at Islamabad (5.2 mmol/m²/s) than at Chakwal (4.7 mmol/m²/s). When comparing the cultivars, a significantly higher transpiration rate (E) was recorded for cv. Sagraone (5.3 mmol/m²/s) and cv. NARC Black (5.0 mmol/m²/s) than for cvs. Kings Ruby (4.7 mmol/m²/s) and Perlette (4.8 mmol/m²/s). The transpiration rate (E) also varied depending on the vine developmental stage: it increased continually until the berry set stage, when it peaked and started to decrease with berry ripening. Overall, a relatively lower transpiration activity was recorded at veraison. The source of variation for PS, L and C was significant; however, it was non-significant (0.05) for the years (Y). Similarly, all the two-way (PS*L, PS*C, PS*Y, L*C, L*Y, C*Y), three-way (PS*L*C, PS*L*Y, PS*C*Y, L*C*Y) and four-way (PS*L*C*Y) interactions varied significantly (Table 3).

7. Water use efficiency (WUE)

The results show that the mean water use efficiency (WUE) varied significantly among the cultivars depending on the phenological stage at both locations during 2019 and 2020. The mean water use efficiency was higher at Islamabad (2.7) compared to Chakwal (1.9) during the second vintage (2.4) in contrast to the previous year (2.1), as given in Table 2. Regarding the individual cultivars, a significantly higher

WUE was recorded for cvs. Perlette (2.4) and NARC Black (2.3), than for cvs. King Ruby (2.1) and Sagraone (2.3). WUE also varied depending on the vine developmental stage, increasing between the vegetative and reproductive phenophases (i.e., accelerated leaf growth to blooming), but it was slightly lower at early berry set and then rapidly decreased during berry ripening. The mean values of WUE at the key phenological stages (i.e., 5 leaf, full bloom, berry set and veraison and harvest) were 1.21, 3.75, 3.35, 1.74 and 1.31 respectively (Table 2 and Figure 9). Furthermore, all four factors and their combinations were significant (Table 3).

8. Multivariate analysis for physiological attributes, cultivars and environments

A principal component analysis (PCA) (a multivariate technique) was carried out in order to determine the relationships between the variables in a high-dimensional dataset. The dataset consisted of the physiological attributes of the grapevine cultivars Kings Ruby, Perlette, Sagraone and NARC Black (Figure 10). The biplot analysis of PC1 and PC2 allow the relationships between the physiological variables and grapevine cultivars to be visualised in a two-dimensional plot. More than eighty percent of total variability was accounted for by the first two principal

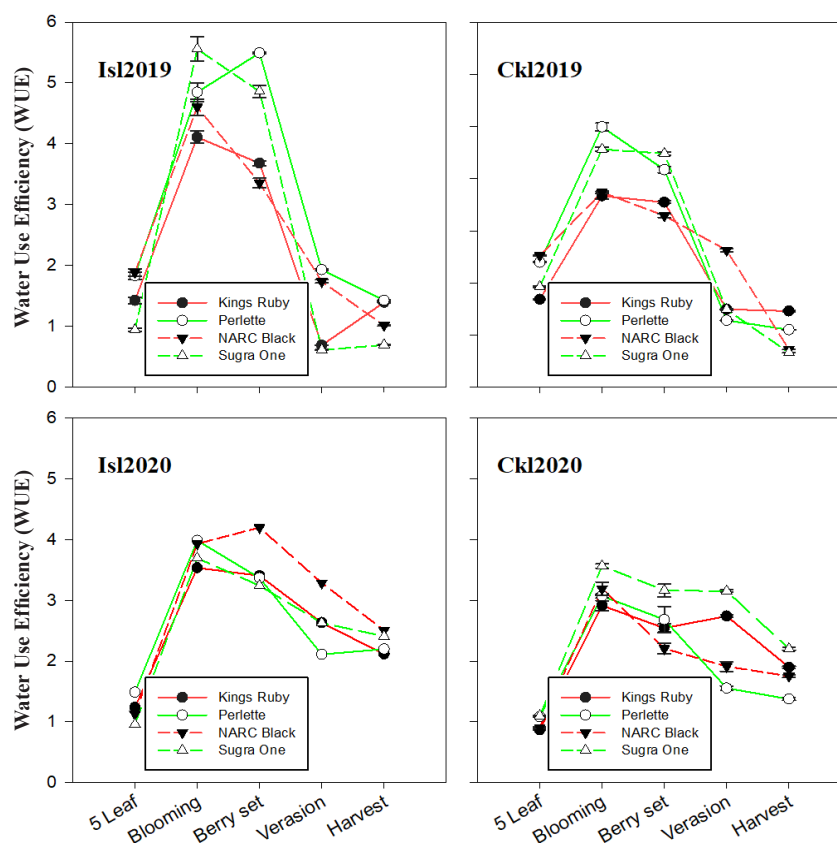


FIGURE 9. Water use efficiency ($\mu\text{mol}/\text{mmol}$) of the four table grape cultivars at key growth stages.

components; i.e., 56.6 % (PC 1) and 26.87 % (PC 2). The PCA shows three distinct groups related to the physiological profiles of the cultivars. The first group contains cv. Sugraone located in the top right-hand quadrant (positive PC), which was highly responsive in terms of transpiration and stomatal conductance. The second group contains Kings Ruby, which is located in the top left-hand quadrant (negative PC1 and positive PC2) and has higher sub-stomatal conductance. Finally, cvs. Perlette and NARC Black in the bottom right-hand quadrant form the third group and have more responsiveness for chlorophyll and WUE. Briefly, the PCA biplot indicates that cv. Sugraone is physiologically efficient and highly responsive for most of the attributes, cvs. NARC Black and Perlette is moderately responsive, and cv. Kings Ruby is the least responsive.

In addition to the cultivars and variables, a PCA was performed to determine the interaction between the physiological attributes and the distinct environments in the two locations (Islamabad and Chakwal) and the two vintages (2019 and 2020). The results show that the first the two principal components account for ninety five percent (84.5 % for PC 1 and 10.8 % for PC 2) of total variability. The biplot analysis indicates that the grapevine physiological activities g_s , E , A & WUE were better regulated in the colder location of Islamabad, particularly in the 2020 vintage (Group 1), whereas the activities related to chlorophyll content and substomatal conductance (Group 2) were better regulated at Chakwal in the 2020 vintage (Figure 11).

However, negative interaction is shown for all the physiological attributes in the hotter vintage of 2019.

DISCUSSION

Leaf chlorophyll content is usually stable; however, it is affected by heat stress, as is leaf growth and other physiological attributes, after a threshold temperature is attained. In comparison to 2019, lower temperatures, higher rainfall and more humid conditions during the 2020 vintage favoured chlorophyll production. These results are in line with a study by Carvalho *et al.* (2015), in which a stepwise heat stress and recovery experimental setup for two wine grape varieties, Tourina Nacional and Trincaderia, resulted in heat stress at 42 °C reducing leaf chlorophyll contents; interestingly, the grapevine leaves tended to recover when exposed to favourable conditions at 25 °C and chlorophyll levels increased in a stepwise accumulation and recovery process. Significantly lower CCI was recorded in the colder location of Islamabad than in Chakwal. This difference was small, but it indicates that, in addition to seasonal variability for temperature, other factors, such as soil type, water and nutrient availability, also contributed to variation in leaf chlorophyll levels (Gruber and Kosegarten, 2002; Sánchez *et al.*, 2020). For instance, soils with higher clay content and lower pH have sub-optimal Fe content, which may negatively affect leaf chlorophyll levels, as was observed in the vineyards of the Mediterranean region (Casanova-Gascón *et al.*, 2018). Similarly, in our case,

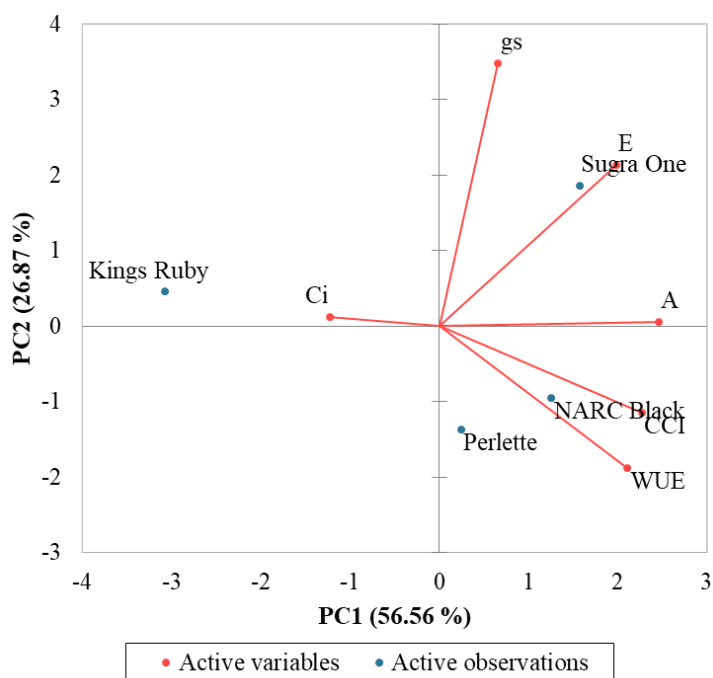


FIGURE 10. Bi-plot of the first (PC1) and second (PC2) principal components of key physiological attributes of the four grapevine cultivars: CCI = Chlorophyll content index, Ci = sub-stomatal conductance, gs = stomatal conductance, A = photosynthetic activity, E = transpiration rate, WUE = water use efficiency.

the soil at Islamabad has a higher clay content than that of Chakwal. During the physiological monitoring of the vines, more vegetative growth was observed in the grapevine cultivars at Islamabad than in those at Chakwal; this might be due to the less favourable climatic conditions in Chakwal, where T max reached up to 40 °C during the summer, negatively affecting vine growth. Given that the same pruning technique was applied to each vine so that 32 to 40 buds per vine remained, further research to evaluate the relationship between leaf chlorophyll and vine vigour, pruning type and bud charge is needed to better understand this behaviour. The lower leaf chlorophyll content observed in Islamabad compared to Chakwal is not likely to have affected the photosynthetic potential of the leaves; Walker *et al.* (2018) found that grapevine canopies can assimilate similar amounts of carbon dioxide with significantly lower leaf chlorophyll levels and can also save up to 9 % of leaf nitrogen. In future studies, it would be useful to explore the relationship between vine vigour and leaf chlorophyll levels and soil nitrogen (N) availability, focusing on the repartitioning of N from chlorophyll towards more beneficial investments in order to accelerate the rate of carboxylation and electron transport reaction. Moreover, different cultivars growing under similar climatic conditions showed varying chlorophyll levels; for instance, cv. NARC Black had the highest leaf chlorophyll content. Such genotypic variability for leaf chlorophyll has been highlighted by Carvalho *et al.* (2015) and Filimon *et al.* (2016): wine grape var. Tourina Nacional

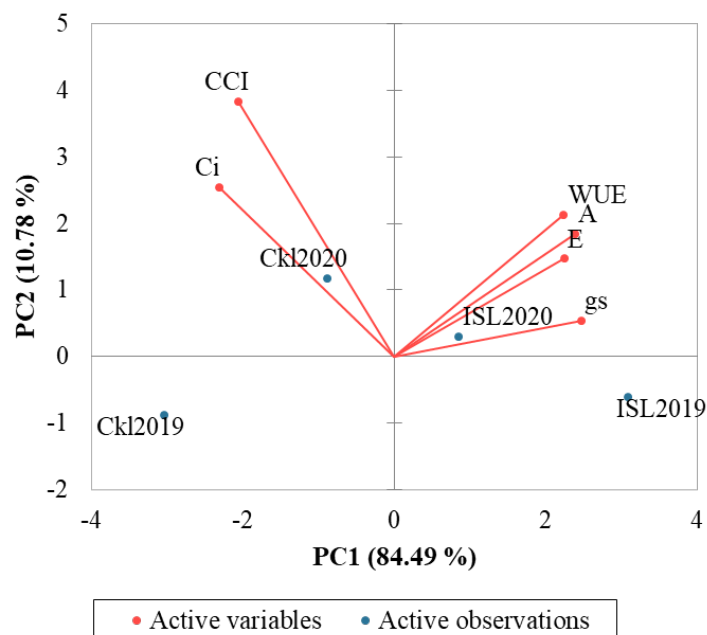


FIGURE 11. Bi-plot graph of the first (PC1) and second (PC2) principal components of the key physiological attributes related to different environments (locations) in 2019 and 2020: CCI = Chlorophyll content index, Ci = sub-stomatal conductance, gs = stomatal conductance, A = photosynthetic activity, E = transpiration rate, WUE = water use efficiency.

contained significantly more chlorophyll than did var. Trincaderia. Moreover, in the present study, CCI also varied depending on phenological stage - from bud burst to maturity - with the highest mean values at harvest. Similar increases in chlorophyll content from bud burst to veraison was observed by Filimon *et al.* (2016) for table grapes var. Gelu, Milcov, Cetatuaia and Napoca. However, in our case, there was a slight increase in chlorophyll content at maturity which might be due to more rainfall near ripening promoting chlorophyll production, even near maturity. In a related study, Chavarria *et al.* (2012) also observed higher chlorophyll content near maturity.

Higher internal leaf CO₂ or sub-stomatal conductance (Ci), particularly for the warmer location (Chakwal) and the hotter vintage (2019), indicate that leaf Ci activity is positively related to temperature. These elevated Ci levels under high temperature conditions are in line with the work of Luo *et al.* (2011), in which higher temperatures (35-45 °C) were found to increase leaf Ci activity. Similarly, Greer (2018) observed that internal CO₂ activity (Ci) was highest at a lower temperature (20 °C), but it decreased at 30 °C and then stabilised at 35 °C. Ci has also been shown to increase at temperatures above 40 °C (Greer, 2012; Greer and Weedon, 2012). The elevated Ci levels at high temperature have a negative relationship with leaf photosynthetic activity (A); this trend is in line with Greer and Weedon (2012b), who also observed a negative relationship between Ci and A under increasingly hot climatic conditions.

In future research on these cultivars, it would be worth focusing on quantifying the C_i and A relationship under the influence of elevated air temperature conditions. In the present study, the internal C_i concentration of the grapevine cultivars also varied, with relatively higher levels being recorded for cvs. NARC Black and King's Ruby compared to Perlette and Sugraone. Similar genotypic variability in grapevine cultivars in different temperature regimes has been highlighted by Greer (2018). It is interesting to observe that the cultivars Kings Ruby and NARC Black, which had significantly higher C_i values, showed visual signs of heat stress, while Perlette and Sugraone, which had lower C_i values, did not show any visual signs of summer heat stress until harvest. In terms of phenological stages, C_i was at its highest at the early developmental growth stages (i.e., $298 \mu\text{mol}/\text{m}^2/\text{s}$ at the 5-leaf stage), and near maturity ($287 \mu\text{mol}/\text{m}^2/\text{s}$); however, the values were significantly lower at the other stages particularly at full bloom and berry set. These results partially correlate with the work of Greer (2012), in which C_i varied during the season with vine growth, being at its highest at the beginning of the season, then decreasing during the season; however, it increased again near maturity.

Leaf stomatal conductance showed high variability between the two locations in the 2019 vintage, which can be linked to their differing temperatures, particularly during the hot summer; meanwhile, there was less leaf stomatal conductance variability between locations in the 2020 vintage. This might be due to the smaller number of days of heat stress in the 2020 vintage, particularly at the warmer location (Chakwal). These findings are in line with those of Luo *et al.* (2011), who observed a decline in stomatal conductance for temperatures above 35°C . In the present study, the stomatal conductance of cv. Sugraone was exceptionally high at Islamabad in 2019, due to higher T_{max} (39°C) on 5 May (berry set) and increased daily evaporation (6.5 mm), compared to the other cases (locations and years) at the onset of the berry set stage, when T_{max} was low (28.5°C to 33°C) with low evaporation (2.0 mm to 5.6 mm). These findings are in line with those of Greer (2012) and Greer and Weedon (2013), who concluded that high temperature above 35°C may likely increase leaf stomatal conductance. In a related study by Sabir and Yazar (2015), stomatal conductance of grapevine cultivars was found to increase from early morning (08:30) to mid-morning (10:30), when a peak value was attained, which then gradually decreased; the stomatal conductance ranged from 96.7 to $287.7 \text{ mmol}/\text{m}^2/\text{s}$. For *Vitis labrusca* var. Niagara and Rosada grown in Brazil, stomatal conductance increased from 8:00 (morning) to 12:00 (noon), but was negative during the afternoon (Deus *et al.*, 2016). Leaf stomatal conductance also varies during the growing season, as reported in this study and discussed by Greer (2018). In another study, Greer (2012) found that stomatal conductance increased from 20 to 25°C , and then stabilised before decreasing at 35°C . However, a further rise in temperature slightly increased stomatal conductance. The stomatal conductance varied significantly between cultivars; similar genotypic variability for leaf stomatal conductance was observed by Sabir and

Yazar (2015) and Greer (2018). Leaf stomatal conductance also varied depending on key phenological stage, with significantly high values at berry set and low values at harvest, which may correspond to the prevailing temperature and plant demand for gaseous exchange at different developmental stages.

Net photosynthetic activity was significantly higher at the relatively cold location of Islamabad than at Chakwal during the colder vintage of 2020. The lower photosynthetic activity for the hotter vintage and colder location indicates the negative effects of high temperature on grapevine physiological processes. These findings are in line with those of Luo *et al.* (2011), who observed that high temperatures above 35°C decreased photosynthesis and that at a temperature of 45°C the acceptor site of PSII was inhibited. Greer (2012) found net photosynthesis to increase with rising temperature from 15 to 35°C , with an optimum range of 25 to 30°C , and to sharply decrease by 30 % (cv. Shiraz) and 60 % (cv. Semilon) at 45°C . Net assimilation in grapevine has been found to peak during the summer at an optimum photosynthesis temperature of $30\text{--}35^\circ\text{C}$ (Greer, 2019; Greer, 2020). Moreover, periods of heat stress can also affect vine photosynthetic responses; for example, heat stress at $25/40^\circ\text{C}$ for 4 days caused a 35 % decline in photosynthesis, with a subsequent 12-day recovery period (Greer and Weston, 2010). Photosynthetic activity varied significantly between cultivars: cvs. NARC Black and Sugraone showed higher photosynthesis values than the other cultivars. Similar genotypic variability for photosynthetic activity has been observed in four table grape cultivars, Crimson seedless, Superior seedless, Razaki and Horoz Karasi (Kamiloglu *et al.*, 2014), in wine grapes cvs. Chardonnay and Merlot (Greer, 2018) and in *Muscadina rotundifolia* var Carlos and Noble in Florida (Colova *et al.*, 2007).

Photosynthetic activity varied depending on key phenological stage, being significantly high at berry set and near blooming stage, but lower at harvest, which may correspond to variable temperature above critical thresholds. Grapevine developmental stages are known to vary in terms of optimum temperature ranges; for example, $25\text{--}30^\circ\text{C}$ is the optimum range for early growth after bud burst, but $30\text{--}35^\circ\text{C}$ is the optimum window for most phenological stages (Greer, 2020). Heat stress negatively affects photosynthetic activity in key phenological stages as observed by Greer and Weston (2010): vine photosynthetic activity decreased from $11 \mu\text{mol}/\text{m}^2/\text{s}$ to $2 \mu\text{mol}/\text{m}^2/\text{s}$ when nearing the maturity stage.

High transpiration is an indicator of an effective photosynthetic process and the plant's ability to perform its physiological processes efficiently. The observed data indicate that the vines at the warmer location (i.e., Chakwal) were subject to conditions of stress (elevated temperature and lower rainfall), which might have caused partial stomatal closure, thus low transpiration. In a related study on *Vitis labrusca* var. Niagara Rosada grown in Brazil, an increase in temperature by 2.3°C significantly increased leaf transpiration at 08:00 (morning), but at 12:00 (noon) negative values for transpiration were obtained due to the higher temperatures (Deus *et al.*, 2016).

While elevated temperatures increase leaf transpiration (Greer, 2012; Greer and Weedon, 2013), they usually act in conjugation with water stress owing to low seasonal rainfall and can hence potentially reduce the transpiration potential of plants in warmer conditions (Kamiloglu *et al.*, 2014). This is because the transpiration rate of grapevine cultivars vary significantly, as was observed in present study. Similarly, Greer (2018) observed 50 % higher transpiration in var. Merlot leaves than in those of var. Chardonnay.

Leaf transpiration rates also varied depending on key phenological stage, being significantly at their highest at berry set and the full bloom stages, possibly due to higher plant demand for gaseous exchange. Moreover, they corresponded to higher temperatures, partial stomatal closure, and relatively low gaseous exchange. Significant variations in leaf transpiration at different growth stages were observed by Andrianasolo *et al.* (2016) in sunflowers. However, the present research findings do not agree with those of Thapa *et al.* (2017), who observed non-significant differences in the transpiration efficiency of sorghum at the six-leaf, flag-leaf, grain-filling and maturity stages. The high sensitivity of the transpiration activity of grapevine compared to other crops could be one reason for these varied responses.

WUE is a critical physiological attribute of vines, reflecting the ratio of carbon assimilated during photosynthesis to transpiration losses for water (Flexas *et al.*, 2010). In the present study, WUE was significantly higher at the colder and more humid location of Islamabad than at Chakwal, and it was higher during the 2020 vintage, which had lower temperatures than in the 2019 vintage. These findings are in line with those of Medrano *et al.* (2015), who observed that high temperatures and water stress negatively affect leaf WUE. The vines managed through the anti-transpirant strategy of applying Kaolin have up to 18 % more WUE, which reduces the adverse effects of heat and water stress, such as sun burn and berry shrivel (Brillante *et al.*, 2016). Genotypic variability among grapevine cultivars affects vine WUE, as was observed in this study, with WUE being significantly higher in cvs. NARC Black and Sagraone than in cv. Kings Ruby. These findings are in line with those of Kamiloglu *et al.* (2014), who also observed WUE to vary in different table grape cultivars. The inherit genetic variability of 23 grapevine genotypes in terms of WUE and stomatal conductance under Mediterranean climatic conditions of Spain (Bota *et al.*, 2016) could be exploited as a means of helping viticulture adapt in the face of future climatic challenges.

Leaf water use efficiency is also significantly affected by crop developmental stages, as was observed in the present study, with WUE being significantly higher at full bloom and berry set stages than at the 5-leaf stage and afterwards near berry ripening. A similar WUE trend has been observed in tomatoes, with significantly low WUE near maturity compared to at other stages, such as vegetative growth, flowering, fruit set, fruit growth and development stages. It has also been highlighted that from a physiological perspective fruit set is a critical growth stage (Xiukang and Yingying,

2016). Furthermore, WUE is affected by crop management practices at key developmental stages (Zhou *et al.*, 2020); therefore, balanced crop management is needed for higher WUE efficiency.

The results of the multivariate PCA analysis revealed discrete genotypic variability among the grapevine cultivars in terms of key physiological attributes; for instance, cv. Sagraone was physiologically more responsive and efficient than the other cultivars. Sabir and Yazar (2015), Carvalho *et al.* (2015) and Greer (2018) have also shown that grapevine cultivars vary in terms of physiological responses under varying environmental conditions. As well as genotypic variability, environmental conditions also highly influence grapevine physiological activity; for instance, the high physiological activity observed in Islamabad in the present study can be associated with the district's colder environmental conditions compared to the warmer conditions of Chakwal, particularly in 2019. These findings are in line with those of Deus *et al.* (2016), Greer (2018) and Greer (2020), who also observed lower physiological activity of grapevines under elevated temperature conditions.

The varied genotypic responses of the grapevine cultivars observed in this study provide an opportunity to select cultivars with physiological adaptations under high temperature regimes. The rise in growing season temperature negatively affected all the grapevine cultivars; a site specific management strategy would thus be necessary to avoid any physiological stress occurring. The results of the present research also highlight the need to apply special management techniques during seasonal heat waves. An adaptation strategy will need to take into account the critical vine developmental stage (i.e., blooming to berry set), as indicated by the results of this research. Comprehensive short-, medium-, and long-term adaptation strategies are necessary for a sustainable viticultural industry. In this regard, short-term measures, such as delaying ripening, scheduling irrigation, applying supplemental irrigation, vine coverings and shading, and use of anti-transpirants (van Leeuwen and Destrac-Irvine, 2017; Caravia *et al.*, 2016; Savi *et al.*, 2018; de Palma *et al.*, 2022) need to be further studied for their effect on vine physiology. Medium- to long-term strategies, such as considering wild rootstocks, selecting physiologically efficient cultivars, introducing characteristics from C-4 plants, and identifying new viticultural zones, could help vines adapt to future climates (Nazir *et al.*, 2022; Rafique *et al.*, 2023ab). Future studies that develop integrated phenology, physiology, yield and fruit quality models using dynamic approaches (Hopf *et al.*, 2022) would be helpful to better understand the complex physiological and biochemical processes of vines.

CONCLUSIONS

Physiological responses of grapevine cultivars are affected by variability in seasonal temperature. The present research findings showed photosynthetic activity, transpiration rate, stomatal conductance and water use efficiency to be higher in the colder location of Islamabad than in

Chakwal, and particularly during the colder vintage of 2020. Of the four cultivars, photosynthetic activity was highest in cvs. Sagraone and NARC Black. The grapevine physiological responses also varied between phenological stages, with peak photosynthesis and transpiration activities being recorded at the berry set stage, and peak WUE at blooming, thus indicating that blooming to berry set is a critical growth period for grapevines. It is evident from the results that the higher growing season temperature near ripening negatively affects photosynthesis, transpiration and leaf water use efficiency, which could reduce yield potential due to impaired photosynthetic processes. Moreover, the physiological assessment of grapevine cultivars provides valuable information for the planning of adaptation strategies to improve vine physiological performance, particularly in warm climates.

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AUTHOR'S CONTRIBUTION

Conception and design of the experiments: RR, TA; carrying out of the experiments RR; drafting of the manuscript: RR, TA and MA; data analysis: RR; Review, editing and preparation of the manuscript for submission: RR, TA, MAK

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