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Water Availability in Pumice, Coir, and Perlite Substrates Regulates Grapevine Growth and Grape Physicochemical Characteristics in Soilless Cultivation of Sugraone and Prime Cultivars (*Vitis vinifera* L.)

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Abstract: Table grape production in soilless cultivation under a controlled environment is a promising solution that addresses many of the challenges of grapevine cultivation, such as factors affecting the quantity and quality of table grape production, cultivation cost, pest management, soil degradation, soil-borne diseases, and adaptation to climate change. However, due to limited knowledge, investigation of many factors is required to effectively implement soilless cultivation, among which are the substrate's physical-hydraulic properties and suitability for grape production. In this context, we investigate the impact of the properties of organic (coir dust) and mineral (perlite, pumice) substrates and their blend (perlite:coir) on grapevine growth and grape physicochemical characteristics of Sugraone (Superior Seedless) and Prime cultivars. Perlite substrate was the best in qualitative and quantitative production characteristics, whereas pumice substrate proved unsuitable for soilless vine cultivation. Coir and perlite:coir substrates, due to their increased ability to retain moisture, improved plant nutrition and grape quality but delayed ripening. For effective soilless cultivation of grapevines and table grape production, substrates must have the ability to maintain sufficient but not excessive moisture, suitable for supporting physiological processes and plant nutrition, resulting in smooth growth and production.

Keywords: table grapes; hydroponics; pumice; coir; perlite

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1. Introduction

Viticulture has been globally recognized for its contribution to the economic, cultural, and social development of rural regions and the formation of their natural environment. However, grapevine growers have to cope with significant emerging problems regarding the factors affecting the grapes' quantity and quality [1], cultivation cost [2], pest management [3], soil degradation [4], soil-borne diseases [5], and adaptation to climate change [5]. Viticulture must address these challenges to become sustainable in the face of climate change and production risks and meet social demand for environmentally friendly cultivation practices.

In an effort to overcome these crucial challenges, viticulture, similar to the rest of the agricultural sector, has taken advantage of the past few decades of rapid scientific, economic, and technological development to achieve dramatic improvements [6]. Cultivations that have been traditionally open-field, such as grapevines [7], fruit trees [8–11], and wild edible greens [12], are gradually replaced by a wide variety of protected cultivation systems, which can significantly increase the production intensity and minimize the risk of production loss due to unpredictable environmental conditions [13].

Additionally, the proliferation of soil-borne pathogens and soil-induced abiotic stress has led to the implementation of soilless cultivation techniques where nutrient and water availability are constantly monitored and adjusted to meet crop-specific demands [14–16]. It is widely established that soilless cultivation systems have increased water and nutrient use efficiency compared to soil-grown plants, resulting in higher yield production per surface area [17]. Recent scientific reports show that soilless cultivation systems can increase the marketable yield of vegetable and ornamental crops by over 25–50%, depending on the plant species [18,19]. Moreover, in the last decade, soilless cultivation systems have been proposed not only for annual crops but also in arboriculture as a promising alternative cultivation technique that can substantially increase water use efficiency and the marketable yield of perennial crops [20]. For instance, Rubio-Asensio, Parra, and Intrigliolo [20] examined a novel soilless cultivation technique for nectarine production in open-field conditions. Their results demonstrate that nectarine trees grown in a substrate with deficit irrigation substantially increased fruit set and prevented fruit drop. Additionally, the results of a greenhouse experiment conducted to assess the impact of fig soilless cultivation under protected conditions demonstrate that yield and functional characteristics significantly increased while minimizing water and nutrient consumption, hence improving the profitability of the proposed cultivation system compared to traditional cultivation methods [21]. Recently, Pisciotta, Barone, and Di Lorenzo [7] showed that soilless table grape cultivation accelerates grape maturity and increases yield and quality characteristics, highlighting the great potential offered by this type of cultivation process. However, studies of hydroponically grown grapes reaching maturity are very limited, leaving several open questions and challenges for wider adaptation by practitioners.

Generally, the soil matrix constitutes a water and nutrient reservoir for plants and provides mechanical support to the roots. In soilless culture, soil physicochemical and mechanical functions are substituted by growing media with ideal hydraulic and physical properties for unrestricted water and nutrient uptake. It is widely established that the proper choice of the most suitable substrate type with desirable physicochemical properties can substantially determine plant growth and, subsequently, the final fruit yield [22]. Through time, several organic and mineral substrates have been proposed (i.e., peat, coir, perlite, and pumice), which successfully replace soil physicochemical characteristics and ensure water availability and root aeration, thus promoting vegetative growth and fruit production. For example, Tangolar et al. [23] reported that the perlite:peat blend significantly increased the marketable yield and cluster weight of the "Early Sweet" grape cultivar compared to the sole cocopeat and basaltic pumice. However, the best yield results for "Trakya ilkeren" and Yalova incisi" grape cultivars were obtained from cocopeat and perlite:peat blends [23]. In contrast, no differences were found among substrates tested in a study evaluating the effect of perlite and its mixtures with attapulgite and zeolite substrates on grapevine leaf production [24]. Nevertheless, knowledge regarding substrate physical-hydraulic properties and their suitability for grape production is limited.

In this context, we examine the impact of pumice, coir, and perlite substrates on grapevine growth and grape physicochemical characteristics of two table grape cultivars in a hydroponic system. The objective is to assess the impact of organic (coir dust) and mineral media (perlite, pumice) and their blend (perlite:coir) on the yield characteristics, mineral concentration, and photosynthetic capacity of the plants. Moreover, substrate moisture and available water content, which affect plant physiology, growth, and grape characteristics, were investigated.

2. Materials and Methods

2.1. Experimental Design

The experiment was conducted in an unheated saddle roof double-span greenhouse covered with polyethylene film with a total area of 180 m^2 (12 m × 15 m) during the growing season of 2022 in the greenhouse facilities of the Hellenic Mediterranean University, Greece. The temperature was kept under 28 °C by automatic ventilation and a fan and pad evaporative cooling system. Two year old Sugraone and Prime grapevines were used for the study, planted in square 11 L plastic pots (26 cm height and 22 cm width) containing four substrates (Table 1) and placed in six specially designed drainage channels spaced 1.5 m apart. Aiming for homogeneity and a balance between vegetation and yield, five to six shoots were left on each vine, supported by a V-type trellis system. Accordingly, four bunches were left per vine after cluster removal at the stage of berry set. Integrated pest management was used to control pests, aided by the controlled conditions of the greenhouse and the insect screens on the windows. A fully randomized design was used in a factorial arrangement of 4 × 2 (substrates × cultivars) and twelve grapevines per substrate for each cultivar (12 × 4 × 2 = 96 experimental units).

Table 1. Substrate treatments and mixing ratios.

Treatment	Mixing Ratios
perlite	100% perlite
pumice	100% pumice
coir	100% coir dust
perlite:coir	50% perlite:50% coir dust

2.2. Plant Nutrition

A modified Hoagland nutrient solution was used (Table 2), adjusted at 1.9 dS m^{-1}, and prepared using the IQ60 (ALAGRO, Athens, Greece) automatic nutrient mixing system. The solution was delivered to the plants via drip irrigation with individual emitters at a flow rate of 2 L h^{-1}. The fraction of the drainage solution released after each irrigation event was maintained within the range of 20–30% by adjusting the frequency and duration in accordance with the climatic conditions. This resulted in three to four daily irrigation applications in each experimental unit. The same irrigation and nutrition management was applied to all four substrates to make their comparative evaluation possible. Air temperature (°C) and relative humidity RH (%) were monitored at 15 min intervals throughout the cultivation period using the RTR-574-S (T&D, Tokyo, Japan) data logging system.

Table 2. Macro- and micronutrient concentrations of the nutrient solution used for grapevine nutrition for all substrate treatments during the experiment.

Macronutrients	Concentration [mmol L^{-1}]	Trace Elements	Concentration [mmol L^{-1}]
NH_4^+-N	1.00	Fe	0.045
K^+	6.00	Mn	0.010
Ca^{2+}	3.00	Zn	0.001
Mg^{2+}	2.00	Cu	0.001
NO_3^--N	14.00	B	0.045
SO_4^{2-}-S	1.17	Mo	0.001
$H_2PO_4^-$-P	1.30		

2.3. Substrate Moisture

Substrate moisture was monitored with GS3 sensors (Meter Group, Inc., Pullman, WA, USA). GS3 sensors use frequency domain reflectometry, taking advantage of the high permittivity of water to estimate the volumetric water content θ [cm^3 cm^{-3}] in the medium [25], over a volume of influence of approximately 400 cm^3 [26]. Under factory calibration, GS3, a generic calibration equation, works in various substrates (e.g., potting soil, perlite, and peat) with an accuracy of better than ±5% cm^3 cm^{-3} [27]. To achieve better accuracy (±1–2%), a media-specific calibration using the standard procedure of Starr and Paltineanu [28] is advised [29]. As shown by Rhie and Kim [30], after substrate-specific calibrations, GS3 measurements are more accurate than other research-grade sensors in measuring volumetric water content of both perlite–coir mixes and sole perlite. Here the calibration procedure was carried out after Starr and Paltineanu [28] by wetting dried (at 105 °C for 24 h) substrate material with tap water at 100 mL (10% cm^3 cm^{-3}) intervals until water holding capacity and thoroughly mixing with a plastic spatula until homogeneous. After each wetting, the substrate was packed around the sensor at hydroponic cultivation bulk density in a 1 L beaker, and measurements were taken for at least ten minutes at one minute intervals. After calibration, eight GS3 sensors were used, one for each substrate for both cultivars, installed in the middle of each pot and fully immersed in the medium. Measurements were stored using the EM50 data logger (Meter Group, Inc., Pullman, WA, USA) at 1 min intervals, and the ECH2O Utility (Meter Group, Inc., Pullman, WA, USA) was used to download data from the logger. Subsequently, available water content [%] was estimated by subtracting substrate-specific moisture content at suction 100 cm (−10.0 kPa or pF 2), commonly used to rate water capacity in substrates (e.g., Gizas et al. [31]), from substrate moisture measurements monitored during the experiment. Moisture content at pF 2 was determined using HYPROP 2 (Meter Group, Inc., Pullman, WA, USA) as described by Shokrana and Ghane [32]. In agreement with Londra et al. [33] and Gizas, Tsirogiannis, Bakea, Mantzos, and Savvas [31], estimated pF 2 values were 13.94%, 19.79%, 20.03%, and 29.16% for the substrate treatments of pumice, perlite, perlite:coir and coir, respectively.

2.4. Physical and Chemical Characteristics of Grapes and Must

The physical and chemical characteristics of grapes were evaluated at harvest time, which was conducted on the same day for all substrates and cultivars for their comparative evaluation. Harvest day was determined based on the Total Soluble Solids (>16° Brix) of the earliest mature grapes, with repeated measurements during the ripening period using a non-destructive Pocket IR Brix Meter PAL-HIKARi 2 (Atago, Tokyo, Japan). For the measurements of grapes and must, the physical and chemical characteristics, four bunches for each substrate and cultivar (n = 32, df = 26) were taken randomly from the main shoots of different grapevines grown at the center of the rows considering the edges as buffer zones. The average values of ten randomly selected berries from each cluster were used for physical characteristics evaluation (weight, width, length, and skin thickness). Berry shatter was estimated by the number of detached berries, after 30 s of vibration at a speed of 500 rpm, of the grape bunches attached by their peduncles on a vibrating arm of a laboratory shaker (Big Bill, Thermolyne; Iowa, USA), and expressed as a percentage of the total number of berries [34]. Total Soluble Solids (°Bx), Total Acidity (g H_2Ta L^{-1}), and Maturation Index (sugar:acid ratio) were estimated using a digital Brix-Acidity Meter PAL-BX | ACID (Atago, Tokyo, Japan).

2.5. Leaf Tissue Analyses

Leaf tissue samples were analyzed for N, P, K, Ca, Mg, Na, Fe, Mn, Cu, and Zn [35]. For nutrient determination, a total of 32 samples, representing four samples for each combination of substrate and cultivar, were analyzed (n = 32, df = 26). The analysis included the examination of petiole tissues during the bloom stage and leaf blades during the veraison stage [36]. Each sample consisted of six leaves (with their petioles), which were randomly collected from the internodes of the basal bunches of three different plants of the same cultivar cultivated on the same substrate. Representative subsamples of the plant material were dried at 65 °C to a constant weight. Subsequently, the dried samples were powdered and passed through a 40-mesh sieve. Total nitrogen concentrations in plant tissues were determined using the Kjeldahl procedure (Gerhardt Kjeldahl KB20 Vapodest®, Königswinter, Germany). The concentrations of Ca, Mg, Fe, Mn, Cu, and Zn were determined using atomic absorption spectrometry (PerkinElmer, Analyst 400). Phosphorus was estimated colorimetrically as phosphomolybdate blue complex at 680 nm using a UV/VIS spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan), while potassium was determined with flame photometry using a Sherwood Model 420 (Sherwood Scientific, Cambridge, UK).

2.6. Phenological Stages

The effect of the different substrates on the promotion of the vegetative cycle of the two cultivars was assessed based on the bud break, bloom, and veraison dates. The bud break date was determined according to OIV code 301, when 50% of the buds were at stage C of Baggiolini. The bloom date was determined according to OIV code 302, when 50% of the flowers were open. According to OIV code 303, the veraison date was determined when about 50% of the grapevines reached the stage where the berries started softening [37].

2.7. Vegetation Characteristics and Physiological Parameters

At veraison, fully expanded leaves between the 10th and 13th nodes of the main shoots of four plants for each substrate and cultivar were chosen to estimate maximum quantum yield efficiency (Fv/Fm) and relative chlorophyll content (SPAD value). Chlorophyll fluorescence (dark-adapted Fv/Fm) has been considered a valuable tool for the relative estimation of the maximum quantum yield of photosystem II photochemistry in several plant species. Additionally, the SPAD value provides an estimation of both leaf chlorophyll content and photosynthetic capacity [38,39]. Chlorophyll content was estimated with a SPAD 502 Plus Chlorophyll Meter (Minolta, Tokyo, Japan), and chlorophyll fluorescence was measured using an OS-30p fluorometer (Opti-Sciences, Hudson, NY, USA) after Baker and Rosenqvist [40] and Jiang et al. [41].

The leaf area index (LAI) was calculated to indicate grapevine vigor during the bloom period. The leaf area of each plant was estimated by measuring the leaf area per shoot (by destructive sampling) and the average number of grapevine shoots [42]. One main shoot was collected at the bloom stage from four plants for each substrate and cultivar ($n = 32$, $df = 26$). All leaves were removed and photographed on a flat white surface with a ruler for scale. At the same time, the average number of shoots was calculated by measuring the number of shoots on all grapevines. The estimation of the leaf area was performed by leaf image analysis using the open-software platform ImageJ 1.53 [43,44]. LAI was calculated as the total leaf area (m^2) of grapevines per unit area of greenhouse floor (m^2).

For the determination of the leaf moisture, all the leaves used for the LAI calculation were used. The sampling was undertaken in the morning, halfway between two irrigations. The leaves were weighed immediately after collection and dried in a laboratory oven (Memmert, Büchenbach, Germany) at 105 °C until they reached a constant weight. The percentage of weight loss was considered as the leaf moisture.

Plant growth and vigor of the grapevines were evaluated based on measurements of plant growth as determined by the length and diameter of shoots [45]. According to the OIV descriptor list for grape varieties and *Vitis* species, at the maturity stage, the length (OIV Code: 353) and the diameter (OIV Code: 354) of ten internodes from the middle third of the first three main shoots of each of the plants ($n = 96$) were measured using a digital caliper [37].

2.8. Statistical Analysis

The data were analyzed using the JMP 17.1.0 statistical software (JMP Statistical Discovery, SAS, Cary, NC, USA). Statistical analysis and graphics were supported using Microsoft Excel 365 (Microsoft, Redmond, WA, USA). Both one-way ANOVA and two-way ANOVA analyses were conducted. The one-way ANOVA was implemented to examine the different interactions among substrates and cultivars and the effects of a single categorical independent variable on a continuous dependent variable. At the same time, the two-way ANOVA assessed the effects of two categorical independent variables. Additionally, the Student's t-test was utilized to compare the means of two groups and determine significant differences between them. Values presented in graphics and tables are treatment means. Significance levels are denoted by symbols: ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. According to the Student's t multiple range test ($p < 0.05$), significant differences among the treatments are indicated by different letters.

3. Results and Discussion

3.1. Substrate Moisture

As shown in Figure 1, the substrate average hourly water content above readily available water content is depicted, indicating a significantly increased percentage of water content above readily available water in coir and perlite:coir compared to perlite and pumice substrate treatments. The results state that the nutrient solution available in the root zone may have been absorbed more by the plants grown in coir and perlite:coir substrate treatments during the irrigation-off hours than those grown in perlite and pumice. Easily available water is a substrate-specific hydraulic characteristic indicating the readily available water content of growing media and is measured to estimate the percentage of available water (%) content that the plants can easily absorb [46,47]. The closer the irrigation regime comes to the threshold of easily available water, the more the plant is at risk of water stress. This hydraulic parameter has been recognized as one of the most crucial substrate properties, indicating growing media's overall performance and suitability across a wide range of vegetable and ornamental crops [48–50].

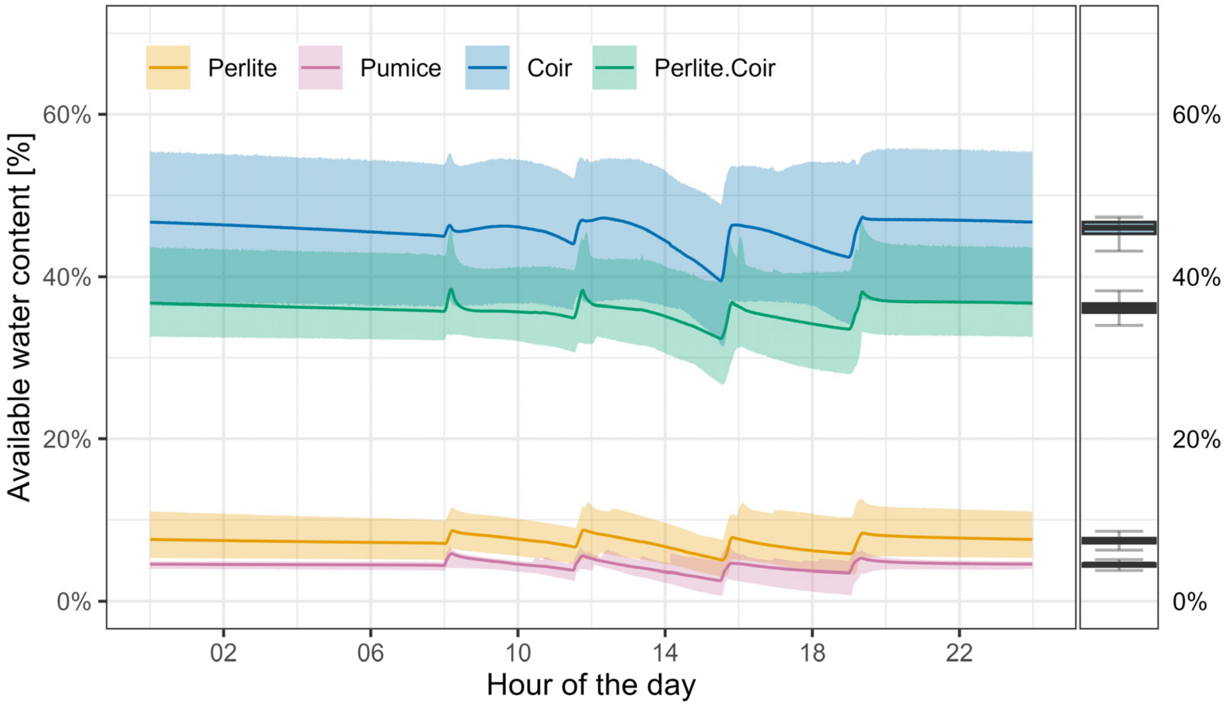

Figure 1. Substrate average hourly water content above easily available water content (%) for the four substrates studied for a ten day interval during the ripening period. The boxplots represent the standard errors of the means. Differences among the substrates are significant according to the Student's t multiple range test ($p < 0.05$).

Correspondingly, Rhie and Kim [30] examined the physical properties of various coir dust and perlite ratios, and their results indicate that substrates with greater perlite content had larger particles, resulting in lower water-holding capacity than substrate mixes with more coir dust. Additionally, according to Gizas, Tsirogiannis, Bakea, Mantzos, and Savvas [31], the hydraulic characteristics of coir and their 1:1 blends (v/v) with pumice significantly affected lettuce growth, which, according to the results of blending coir with pumice, reduced the rate of unsaturated hydraulic conductivity (K_r) compared to 100% coir, highlighting that the differences in the mean fresh weight between lettuce plants were similar to those in the rate of K_r decrease with increasing suction. Hence, the crucial factor for the yield performance of lettuce was water availability, which depends on water flux toward roots and concomitantly on the hydraulic conductivity of the substrate.

3.2. Physical and Chemical Characteristics of Grapes and Must

Results indicate that cultivation on different organic and inorganic substrates significantly affected the most measured physical and chemical characteristics of grapes for both cultivars (Table 3). As shown in Table 3, cultivation on perlite, coir, and perlite:coir substrates increased bunch weight, rachis weight, and berry length compared to pumice substrates. An induced promotion of ripening was observed on the perlite substrate, demonstrating higher sugar content and, thus, a higher maturation index than the other substrates. These results can be attributed to the moderate water deficit imposed by perlite, which limits plant vigor without having any adverse effect on the plant's photosynthetic capacity or nutrient status. In contrast, the limited water availability in the pumice substrate and the excessive water availability in the coir and perlite:coir substrates resulted in delayed ripening [51]. Additionally, the delayed maturation of grapes can partially be attributed to sodium accumulation in the leaves of coir and perlite:coir treatments. High sodium uptake concentration levels can disrupt the absorption and availability of other macronutrients in the grapevine, presumably due to an antagonistic interaction with sodium and chlorine in uptake sites [12,52]. This antagonistic effect of sodium leads to nutrient imbalances and decreased absorption of several mineral macronutrients, including potassium, calcium, and magnesium, which are vital mineral nutrients for the fruit ripening process [53–55].

Table 3. Effect of substrates on the physical and chemical characteristics of grapes and must. Values are treatment means; significance level (Sig.): ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. The different letters among the treatments indicate significant differences according to the Student's t multiple range test ($p < 0.05$).

	Substrate					Cultivar			Interaction
	Perlite	**Pumice**	**Coir**	**Perlite:Coir**	**Sig.**	**Sugraone**	**Prime**	**Sig.**	**Sig.**
Bunch Length (cm)	27.55 a	27.54 a	30.11 a	28.89 a	ns	26.59 b	30.46 a	**	ns
Bunch Width (cm)	16.67 b	18.64 ab	21.50 a	18.71 ab	*	18.32 a	19.44 a	ns	ns
Bunch Weight (g)	597.79 a	414.50 b	578.85 a	591.05 a	*	589.33 a	501.77 a	ns	ns
Rachis Length (cm)	25.87 a	25.26 a	26.75 a	25.85 a	ns	23.87 b	27.97 a	***	*
Rachis Weight (g)	11.14 a	5.80 b	10.66 a	9.16 a	***	9.29 a	9.09 a	ns	ns
Berries Amount	141.20 a	109.25 a	135.75 a	137.10 a	ns	111.35 b	150.30 a	**	ns
Small Berries Amount	9.17 a	8.00 a	8.50 a	3.00 b	*	2.67 b	11.67 a	***	*
Bunch Density	5.37 a	4.29 b	5.05 ab	5.24 a	ns	4.66 b	5.31 a	*	**
Berry Shatter (%)	0.92 b	2.16 a	1.08 b	1.21 b	*	0.41 b	2.28 a	***	**
Berry Weight (g)	4.43 a	4.02 a	4.32 a	4.33 a	ns	5.27 a	3.28 b	***	**
Berry Length (mm)	24.49 a	20.76 b	25.15 a	24.67 a	***	25.82 a	21.72 b	***	**
Berry Width (mm)	19.30 a	16.97 c	18.47 b	18.76 ab	***	19.57 a	17.18 b	***	***
Skin Thickness (mm)	0.27 ab	0.31 a	0.22 bc	0.19 c	***	0.28 a	0.21 b	***	ns
TSS (°Bx)	17.62 a	11.26 c	12.89 b	12.91 b	***	12.77 b	14.57 a	***	***
TA (g H2Ta/L)	6.19 a	6.39 a	6.43 a	5.82 a	ns	6.89 a	5.53 b	***	ns
Maturity Index	29.03 a	17.98 c	20.71 bc	22.80 b	***	18.69 b	26.57 a	***	*
pH	3.86 a	3.65 b	3.82 a	3.75 ab	*	3.63 b	3.91 a	***	*

The improvement of the qualitative and quantitative grape characteristics in the perlite, coir, and perlite:coir substrates was affected by sufficient water availability, adequate photosynthetic capacity due to the high LAI [56], and proper plant nutrition. Adequate nitrogen uptake and sufficient phosphorus and potassium absorption (Table 4) during the critical period of bloom and veraison have been frequently documented to promote grapevine plants' growth and yield performance [57–60].

Table 4. Effect of substrates on macro- and micro-elements nutrient status of grapevine leaves. Values are treatments means; significance level (Sig.): ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. The different letters among the treatments indicate significant differences according to the Student's t multiple range test ($p < 0.05$).

	Time	Substrate					Cultivar			Interaction
		Perlite	Pumice	Coir	Perlite:Coir	Sig.	Sugraone	Prime	Sig.	Sig.
N (g/kg)	Bloom	43.8 a	42.30 b	41.80 b	43.80 a	**	45.20 a	40.70 b	***	***
	Veraison	38.00 a	36.90 b	36.70 b	38.40 a	**	38.20 a	36.70 b	***	***
P (g/kg)	Bloom	1.56 b	1.32 c	2.20 a	2.08 a	***	1.49 b	2.09 a	***	***
	Veraison	2.40 b	2.62 b	3.37 a	3.37 a	***	2.80 b	3.09 a	*	***
K (g/kg)	Bloom	12.80 c	12.10 c	16.40 a	12.10 c	***	15.50 a	12.60 b	***	*
	Veraison	9.90 c	9.00 d	14.80 a	13.10 b	***	12.60 a	10.80 b	***	***
Ca (g/kg)	Bloom	8.70 a	8.30 a	8.50 a	8.30 a	ns	8.40 a	8.50 b	ns	ns
	Veraison	4.80 b	6.00 a	3.60 c	5.00 b	***	4.80 a	4.90 a	ns	***
Mg (g/kg)	Bloom	2.20 c	2.40 c	2.80 b	3.10 a	***	2.60 a	2.70 a	ns	***
	Veraison	2.60 c	3.10 b	3.10 b	3.30 a	***	3.20 a	2.90 b	***	***
Na (g/kg)	Bloom	2.80 c	2.60 c	4.00 a	3.60 b	***	3.70 a	2.80 b	***	**
	Veraison	2.40 c	2.20 c	3.80 a	3.40 b	***	3.30 a	2.60 b	***	***
Fe (mg/kg)	Bloom	111.20 b	117.55 b	151.95 a	143.58 a	***	146.83 a	115.32 b	***	***
	Veraison	123.15 a	105.30 b	117.03 ab	109.30 ab	*	133.93 a	93.46 b	***	*
Mn (mg/kg)	Bloom	176.15 a	128.28 b	175.53 a	152.68 a	**	149.45 b	166.86 a	*	***
	Veraison	281.86 a	133.20 c	222.12 b	206.13 b	***	201.08 a	220.57 a	ns	***
Cu (mg/kg)	Bloom	1.23 bc	0.35 c	3.03 a	1.88 ab	**	3.19 a	0.05 b	***	**
	Veraison	0.00 c	1.21 b	0.00 c	2.53 a	***	0.60 b	1.27 a	*	***
Zn (mg/kg)	Bloom	14.87 c	18.00 ab	19.76 a	17.81 b	***	18.08 a	17.14 a	ns	**
	Veraison	16.02 a	15.79 a	16.98 a	16.93 a	ns	14.96 b	17.90 a	***	**

In contrast, reduced yield characteristics occurred in pumice-grown plants, which may be ascribed to the limited partitioning of photosynthetic assimilates as a result of the low LAI [56], the low water holding capacity, and hence the reduced plant nutrient uptake since water is essential for nutrient transport from the roots [61]. Indeed, a significantly greater thickness of the berries' skin was observed in the pumice substrate due to the decreased water availability of the substrate (Table 3). This is in accordance with Porro et al. [62] and Zsófi et al. [63], who reported a significant increase in berry skin thickness under water stress conditions. Moreover, increased skin thickness could be affected by high calcium concentrations during the ripening period, which may lead to thicker epidermal and hypodermical layers and increased skin resistance [64,65]. The increased skin thickness can increase grapes' resistance to fungi, both pre-harvest and post-harvest [66]. However, the hardness and texture of thick skin could negatively affect consumer acceptance of the product [67]. Moreover, due to reduced moisture sufficiency, a significantly greater berry shatter was observed in the pumice substrate, a quality factor that also reduces consumer acceptance [68].

Accordingly, significant differences were also observed between the two cultivars in the physical and chemical characteristics of grapes and must, with the Prime cultivar demonstrating a higher maturation index, bunch length, and berry amount compared to Sugraone, and vice versa for berry length and width (Table 3).

Nonetheless, the difference in maturity between the two cultivars was prospective, as the Prime cultivar usually ripens almost two weeks earlier than Sugraone [69]. Furthermore, the interaction between the substrates and cultivars revealed an intriguing interplay influenced by the distinct characteristics of each cultivar.

Sugraone grapes grown in a coir substrate showed a noteworthy increase in bunch weight, density, and the number of berries. In contrast, Prime grapes displayed significantly improved physical characteristics when cultivated on a perlite substrate (data not shown). Even though the statistical analysis showed a small number of interactions between substrate treatments and cultivars (for some of the characteristics studied), all such interactions were ignored since there were no changes in rank, and only the main effects are presented in the tables.

3.3. Macro- and Micro-Elements Nutrient Status of Grapevine Leaves

As revealed by leaf tissue analysis, Na^+ concentration was significantly higher in plants grown on coir substrate compared to perlite:coir, pumice, and perlite substrate treatments (Table 4). Coir is an organic substrate with high ion exchange capacity due to the presence of polysaccharides in the organic matter of the growing medium [70]. Thus, the main cations adsorbed to the fixed negative charge of coir are mainly K^+ and Na^+, which probably explains the increased uptake of Na^+ from the plants compared to the inorganic growing media of perlite and pumice. Contrarily, on coir substrates, the adsorption of Ca^{2+} and Mg^{2+} is relatively inadequate, leading to nutritional imbalances in the plants [71,72]. In agreement with this consideration, at veraison, leaf Ca^{2+} concentration was significantly reduced in coir compared to pumice, perlite, and perlite:coir mixture substrate treatments. However, Mg^{2+} concentration was significantly reduced in sole perlite compared to all the rest of the substrate treatments.

Additionally, K^+ and phosphorus concentrations were significantly increased in sole coir and perlite:coir blends, which can be related to the increased available water content and concomitantly to the increased nutrient uptake of the plants compared to sole perlite and pumice growing media (Figure 1). However, the organic-N concentration of the leaves was reduced in pumice and coir substrates, presumably as a result of the decreased nitrate nitrogen uptake due to the reduced available water content of the former and the increased Na^+ and Cl^- absorption of the latter and their antagonistic interaction with nitrate nitrogen in uptake sites [73]. Another intriguing result is that, considering the effect of substrate type on Cu trace element absorption at veraison, leaf copper concentration was significantly increased in the perlite:coir blend compared to sole perlite and coir substrate treatments, presumably as a result of the increased Fe and Mn absorption, which compete with Cu in uptake sites, leading to reduced leaf copper concentration at veraison [74]. Correspondingly, Serpil et al. [75] reported that leaf Cu concentration was significantly increased in the zeolite:cocopeat blend compared to sole zeolite and cocopeat substrate treatments in soilless Cardinal grape cultivation. Furthermore, significant variations were observed in the nutrient profiles among the two cultivars, with significant interactions detected between the substrate and cultivar variables across nearly all examined nutrients. These disparities primarily arise from the distinct genetic traits inherent to each cultivar, exerting substantial influence on their capacity to support plant nutrition [51]. Consequently, when choosing an optimal substrate, careful consideration must be given to the specific attributes of the targeted cultivar to be grown. Nonetheless, despite the differences observed in the macro- and micro-element status of the plants, macronutrient and trace element concentrations in the leaves were within the optimal range in all treatments, and none of the tested media exhibited any visible symptoms of macronutrient or trace element deficiency during the cultivation period [76].

3.4. Phenological Stages

Cultivation on the different substrates, as revealed by two-way analysis, significantly affected the date of bud break, with the pumice substrate causing an earlier bud break than the other substrates (Table 5) at a statistically significant number of days ($\approx$7.5). A similar promotion was also observed in the bloom stage and the veraison, with the pumice substrate causing a six day earlier flowering and a 3.4 day earlier veraison compared to the rest of the substrates. However, the low water availability, inadequate photosynthetic capacity, and inappropriate nutrition in the pumice substrate, as discussed above, resulted in the suspension of the promotion during the ripening period, which resulted in the pumice substrate having the latest maturation compared to the rest of the substrates (Table 1). This is in agreement with Smart and Coombe [77], who reported that moderate water stress stimulates the vegetative cycle, from bud break to veraison, but delays ripening. Moreover, a significantly earlier bud break occurred in the Prime cultivar than in Sugraone. This is probably due to the earliness of the Prime cultivar [69] and agrees with previous studies suggesting that the cultivar's genotype highly influences bud burst time [78–80] (Table 5).

Table 5. Substrates effect on the phenological stages of grapevines in a soilless cultivation system. Values are days after the first day of the year. Significance level (Sig.): ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. The different letters among the treatments indicate significant differences according to the Student's t multiple range test ($p < 0.05$).

	Substrate					Cultivar			Interaction
	Perlite	Pumice	Coir	Perlite:Coir	Sig.	Sugraone	Prime	Sig.	Sig.
Bud Break	74.92 a	67.46 b	77.04 a	73.00 a	**	79.85 a	66.35 b	***	ns
Bloom	129.13 a	123.13 b	130.13 a	128.17 a	*	126.85 a	128.42 a	ns	ns
Veraison	169.79 a	166.88 b	170.58 a	170.42 a	**	169.29 a	169.54 a	ns	ns

3.5. Vegetation Characteristics and Physiological Parameters

As shown in Table 6, substrate treatments did not affect maximum quantum yield efficiency (Fv/Fm) or chlorophyll relative content (SPAD). However, both Fv/Fm and SPAD relative concentrations were significantly increased in the Sugraone compared to the Prime cultivar. The SPAD index measures the absorbance of a leaf in the red and near-infrared regions and estimates the relative chlorophyll concentration of the leaves. The maximum quantum efficiency of PSII photochemistry (Fv/Fm) has been extensively used to detect stress-induced perturbations in the photosynthetic apparatus [39,81]. Nonetheless, no significant differences were observed among substrate treatments regarding SPAD values and Fv/Fm measurements, which presumably indicates the lack of osmotic stress-induced changes in the photosynthetic capacity of the plants related to the water availability of the substrate treatments used in our experiment.

Table 6. Interaction effects of substrate treatments and cultivars on vegetation characteristics and physiological parameters of grapevines in a soilless cultivation system. Significance level: ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. The different letters among the treatments indicate significant differences according to the Student's t multiple range test ($p < 0.05$).

	Substrate					Cultivar			Interaction
	Perlite	Pumice	Coir	Perlite:Coir	Sig.	Sugraone	Prime	Sig.	Sig.
Quantum Yield Efficiency (Fv/Fm)	0.81 a	0.79 a	0.80 a	0.80 a	ns	0.81 a	0.79 b	**	ns
Chlorophyll Relative Content (SPAD)	36.20 a	40.20 a	38.21 a	36.10 a	ns	39.38 a	35.98 b	*	ns
Leaf Area Index (LAI)	3.20 a	2.47 b	2.25 b	3.23 a	**	2.71 a	2.87 a	ns	***
Leaf Moisture (%)	82.85 b	83.30 b	85.52 a	84.31 ab	**	0.85 a	0.83 b	***	ns
Internodes Diameter (mm)	8.33 a	7.95 b	8.43 a	8.02 b	***	9.02 a	7.35 b	***	***

The leaf area is a crucial indicator of nitrogen use efficiency and has a considerable impact on plant yield parameters [52]. Sole perlite and blending perlite with coir (50:50) significantly increased the LAI compared to sole coir and pumice. The results can be partially attributed to the increased water availability of perlite:coir and perlite compared to pumice, but mainly to the significantly increased organic nitrogen (%) concentration of the leaves in perlite:coir and perlite compared to pumice and sole coir. Correspondingly, Moschou et al. [82] investigated the effect of grocery waste compost on the growth parameters of lettuce plants, and according to the results, grocery waste-based compost led to a significant increase in leaf area (cm^2) compared to perlite and coir substrate.

Leaf moisture was significantly higher in the grapevines grown on coir substrates compared to perlite and pumice substrates (Table 6), because of the higher readily available water content (Figure 1). Moreover, significant variations were observed in internode diameters, with substrates such as perlite and coir having the most significant positive effect (Table 6). Regarding the different cultivars, Sugraone was found to have significantly higher values than Prime in all the vegetation characteristics and physiological parameters determined, except for the LAI, where the results were similar between the two cultivars (Table 6).

4. Conclusions

The study has provided valuable insights into the impact of pumice, coir, and perlite substrates on grapevine growth and grape physicochemical characteristics of two grapevine cultivars, Sugraone and Prime, in soilless cultivation. The findings highlight the crucial role of substrate selection in achieving optimal growth and production in grapevines. This is primarily achieved by ensuring sufficient moisture and a readily available plant water supply. Adequate water content is particularly important as it directly affects the plants' ability to absorb nutrients effectively.

Given its ability to support high-quality and high-yielding crops, perlite emerges as the optimal substrate for soilless grapevine cultivation, promising enhanced agricultural outcomes and the potential for greater profits. Using perlite resulted in both cultivars' highest total yield, marketable yield, fruit size, and soluble solids content. In contrast, although the pumice substrate had a significant effect on early bud break, bloom, and veraison, it proved unsuitable for soilless vine cultivation due to its limited ability to retain moisture and support plant nutrition, resulting in low quality and quantity of production. Coir and perlite:coir substrates, due to their increased ability to retain moisture, improved plant nutrition and grape quality but delayed ripening.

In summary, successful soilless cultivation of grapevines relies on substrates that maintain appropriate moisture levels, foster physiological processes, and provide plant nutrition for optimal growth and production. Perlite substrates show promise for enhancing table grape production and overcoming conventional cultivation issues. Further research can refine soilless practices and explore alternative substrates to advance grapevine cultivation even more.

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