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Impact of pruning severity and training systems on red and white seedless table grape (*Vitis vinifera*) qualitative indices

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Abstract

This study was carried out to evaluate the effect of bud pruning severity and training systems on some of the pomological traits of red and white seedless table grapes cvs white seedless and red seedless. The 12-year-old vines were trained in conventional and trellis systems each pruned to 2, 6 and 8 buds in canes. Results revealed that soluble solid content was significantly affected (24.2 °Brix) by the training system and bud pruning, whereas bud pruning had no significant effect on titratable acidity. Fruits from the trellis training system had a higher total phenolic content, total flavonoids and flavonoid compounds content as compared with conventional system. In white seedless cultivar, the highest antioxidant capacity was found in 4 buds pruning of trellis system (77.3%), while in the red cultivar 8 buds pruning of trellis system had the highest antioxidant capacity (78.7%). Overall, trellis training system with moderate bud pruning can be considered for increasing table grape nutritional quality.

Keywords: Grapes, Trellis system, berry quality, Phenolics, Anthocyanin, Quercetin.
Abbreviations: HPLC_High Performance Liquid Chromatography; DPPH_2, 2-diphenyl-1-picrylhydrazyl, SSC_Soluble solid content; TA_Titratable acidity.

Introduction

Grape is one of the fruit crops most widely grown in many areas of the world due to its economic importance and the beneficial effects on human health,. In table grape cultivars the berry size, firmness, sweetness and color are important variables as shown by Williams et al. (2010). The cultivation of seedless red and/or white table grape cultivars have increased considerably in the last decades since consumers of many countries appreciate very much the lack of seeds in the berries and the firmness and sweetness of these varieties. This variety has excellent eating characteristics; berry texture is firm and crisp, and its flavor is sweet and excellent (Faci et al., 2014). Consumer awareness of the relationship between foods and health, together with environmental concerns, has led to an increased demand for foods with high nutritional qualities. Due to grape is a phenol-rich fruit, widely consumed in different forms as fresh, raisins, vinegar, molasses, grape juice, etc. (Weston, 2005). A wide range of biological activities of these phenolic compounds has recently been reported: inhibition oxidation of human low-density lipoproteins, antioxidant properties and radio-protective effects, prevention of cataract, anti-hyperglycemic effects, modulation of the expression of antioxidant enzyme systems, anti-inflammatory effects and therapy of cancer (Xu et al., 2010). According to the results of Zheng and Wang (2001) phenolic compounds and especially flavonoids are the main components of antioxidant system in plants. Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics. The quercetin and catechin are the subjects of many studies investigating the biological effects of flavonoids, because these two are the predominant flavonoids found in grape (Iacopini et al., 2008). The polyphenolic compounds content can be mainly influenced by grape variety, berry ripening degree, tissue type (flesh or peel), environmental conditions (biotic or abiotic stress), cultural practices including crop load, thinning and fertilization conditions, together with the vine growing methods – specifically, the pruning and training systems – used (Coletta et al., 2014; Yuri et al., 2012). The architecture of the plants conditions the interception of the light in the canopy and the relations between the leaves and the fruits. It has been widely reported that trellis systems dividing vine canopy increase sunlight penetration, which in turn increase yield and fruit quality. Benefits from a more efficient use of sunlight, the only energy source freely available to growers, influencing photosynthetic rates, bud differentiation, berry size, color and sugar content, fruitfulness, fruit ripening, fruit composition and hardiness of buds and canes (Vanden-Heuvel et al., 2002). The trellising of grapevines, a common practice in the vineyard world, leads to alterations in growth, yield and fruit composition through inter alia its influence on the microclimate, which helps the growers to employ management techniques that improve production quality (González-Neves et al., 2004). While tradition may have a bearing on the predominant training system used in a particular area, the final choice is often determined by the efficiency of the training system producing fruits of a desired quality (Reynolds and Heuvel, 2009). It should also be noted that to choose the best training system for cultivating grapes the following criteria need to be met: (a) trellis provide possible manipulations to retard and avoid drought damage during short dry periods, regulate reproduction growth and vegetative growth and control disease; (b) a simple, low cost technique for modifying the microenvironment of fruit development and shortening the transportation distance of nutrient between root and berry to improve fruit quality, facilitate sufficient maturity and precocity of all the berries under cooler climatic conditions; and (c) a trellis design and training techniques to improve harvest efficiency by maintaining fruiting zone to a accordant low position and be compatible with the winter protection strategy (Nan et al., 2013). Ideally, training systems should be labor-efficient and adapted to the local climate (Cavallo et al., 2001), to mitigate conditions that are unfavorable for growth and promote for the objectives of guaranteeing high quality of grapes, stable yield, longevity and beauty of the vineyards (Li and Fang, 2005). Pruning, one of the cultural operations carried out in the vineyards, has important implications for vine function as it influences the form and size of the vine, the balance between vegetative and fruit growth in the vine, the quantity and quality of grape production. The ratio between the vegetative organs and crop weight has crucial importance for grape quality, and that ratio can be affected through different numbers of buds retained at pruning, the variation in shoot numbers, and defoliation, or by affecting the cluster number per vine (Tassie and Freeman, 2001). In many areas, various pruning systems are used for table grape cultivars, namely spur (2–3 buds), half-cane (6–8 buds) and cane (14–16 buds) systems, depending on the cultivar and region, as fruitful cultivars are spur pruned while less fruitful cultivars are half-cane or cane pruned (Lombard et al., 2006). An average vine before pruning may have 25 canes and 750 buds (Winkler et al., 1997), and it is too important to practice removal of some of them from the vine for the best quality grapes. However, few comparative studies have been carried out concerning the changes in nutritional quality of grape response to different pruning and training systems. They have mainly focused on the yield, yield components and morphological attributes. Therefore, this study is aimed to evaluate the effects of different pruning and training systems on physicochemical characteristics especially polyphenolic compounds concentration and total antioxidant capacity of white and red seedless table grape.

Results and discussion

Soluble solid content (SSC), Titratable acidity (TA) and SSC/TA ratio

The results showed that the pruning and training system significantly affected the SSC (Table 2). In white seedless grapes the highest SSC was found in 6 buds pruning of conventional training system, while in red seedless cultivar the highest SSC was found in 8 buds pruning of trellis training system. As long canes produce more vegetative units with more leaves as photosynthetic organs, they could be potential sources of carbohydrates for berries as demanding sinks. These results are in agreement with the findings of Reynolds et al. (2004) and Terence (2008), who found that pruning and training systems had the significant effects on SSC. Flower bud formation in grape vine occurs in one-year shoots staring from mid-summer along with current fruit growth and ripening. The more the shoot bearing capacity, the higher the flower bud formation would be. Hence, pruning enhances new vegetative growth leading to higher photosyntate accumulation. Since the good training has a dominant influence on the amount of leaf area exposure, the optimal leaf area/crop weight ratio of training systems could explain the results obtained here. Furthermore, Kiliewer and Dokoozlian, (2005) reported that divided canopy-training systems have considerably higher percentage of their leaf area at light saturation compared to that of single canopy-training systems, and therefore a lower leaf area/fruit weight ratio to mature fruit would be expected. Generally, berries grown in full sun compared to partial sun had the higher soluble solids content (Ristic et al., 2007). Both white and red table grape cultivars showed similar TA content by pruning treatment (Table 2), whereas training systems had significant effect on TA. Vian et al. (2006) reported that acidity was not strongly affected by different levels of pruning in the ‘Shiraz’ and ‘Riesling’ grape cultivars. According to Smart and Robinson (1991), the incidence of sunlight into bunches contributes to changes in organic acids content. As change in SSC and TA content, SSC/TA ratio also significantly affected by training systems. Moreover, Uslu and Samanci (1998) reported significant effects of different training methods on TSS/acidity values in the ‘Beyaz Çavus’ and ‘Hamburg Misketi’ cultivars.

Total phenolics, flavonoids and flavonoid compounds

In general, changes in total phenolics content, total flavonoids (Table 2) and flavonoid compounds by different training and pruning methods displayed similar patterns (Fig. 1 and 2). It was found that trellis training system led to higher total phenolics content, total flavonoids and flavonoid compounds as compared with conventional system, with an exception in white seedless cultivar that the highest quercetin content was obtained in conventional system with 6 buds pruning treatment (Fig. 1b). It was found that trellis system increase sunlight penetration, which in turn increase yield and fruit quality. Previous research has found a relationship between canopy structure and sunlight exposure and subsequent fruit polyphenolics compounds (Mabrouk and Sinoquet, 1998). Light influences the growth and composition of a wide variety of fruit, including grapes. Grape berries exposed to sunlight are generally higher in total phenolics Dokoozlian and Kliewer (1996). An increase in the content of quercetin in favorable light conditions compared to shaded clusters was determined by Price et al. (1995) and Downey et al. 2006. Adams (2006) also reported that in the red wine cultivars, the amount of flavonols was highly dependent on light exposure of the tissues in which they accumulated. Plants are potential sources of natural bioactive compounds such as secondary metabolites. They absorb the sun light and produce high levels of oxygen and secondary metabolites by photosynthesis. Flavonoids and phenolics are most important groups of secondary metabolites and bioactive compounds in plants (Kim et al., 2003). Previous studies suggested that some flavonoids such as catechin and quercetin could be able to control cancer cell growth in the human body (Arts et al., 2002; Davis et al., 2000). Phenolics and flavonoids synthesis in plants is influenced by environmental factors. Light is one of the most important environmental stimuli, not only involved in the regulation of plant growth and organogenesis, but also in the biosynthesis of plant products including both primary and secondary metabolites (Ghasemzadeh et al., 2010). Phenolic biosynthesis requires light or is enhanced by light, and flavonoid formation is absolutely light dependent and its biosynthetic rate is related to light intensity and density (Xie and Wang, 2006). However, different plants had a different response to light intensity alteration and the resulting total flavonoids and total phenolics production. It was revealed that changes in light intensity -with changes in plant morphology and physiological characteristics- were able to change the production of flavonoids and phenolics in plants (Ghasemzadeh et al., 2010).

Table 1. Meteorological and pedological situation of the red and with seedless grapes' vineyard in the experimental orchard in Takestan region, Qazvin-Iran.

Parameters	Indices/Values
Latitude	36˚ 03' N
Longitude	49˚ 42 E
Elevation above sea level	1275 m
Soil texture	Loam
Soil/Water EC	1.17 ds m^{-1}
Soil /Water pH	7.6
Relative humidity	53%
Mean of yearly precipitation	120 mm

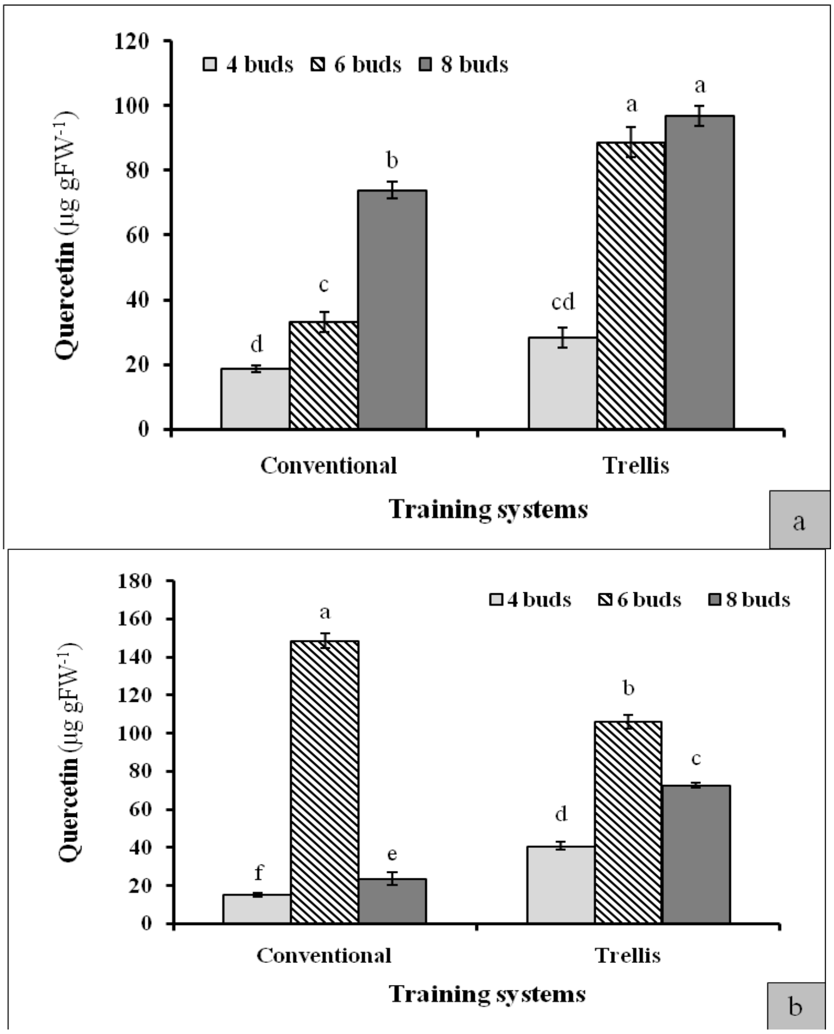

Fig 1. Effects of training systems and bud pruning on the quercetin content of red (a) and white (b) seedless table grapes. The values are the means ± SE. Indicate the test and p level.

Polyphenols are synthesized from the phenylalanine produced by the shikimic acid pathway. The deamination of phenylalanine catalyzed by enzyme phenylalanine ammonia-lyase (PAL) is the first step in this biosynthetic pathway. PAL is induced by a variety of stimuli such as light irradiation (Ruiz-García and Gómez-Plaza, 2013). Moreover, when plant cells were exposed to higher light intensity, increasing activity of special enzymes in pentose phosphate pathway (PPP) acting to provide precursors for phenolic synthesis (Xu et al., 2007). The difference in total phenolics content, total flavonoids and flavonoid compounds between pruning treatments was probably due to the difference in the yield per vine. This agrees with the results of Zhao et al. (2006) for cv. Cabernet Sauvignon vines with a varying crop level.

Antioxidant capacity

As shown in table 2, training systems and pruning treatment had significant effects on antioxidant capacity of white and red seedless grapes. In general, trellis training system had the higher antioxidant capacity than conventional system. In white seedless cultivar, the highest antioxidant capacity was found in 4 buds pruning of trellis system, while in red seedless cultivar 8 buds pruning of trellis system had the highest antioxidant capacity (Table 2).

Table 2. Effects of different training systems and bud pruning on physicochemical composition of red and white seedless table grape.

Cultivars	Training systems	Pruning	SSC (°Brix)	TA (%)	SSC/TA	Total phenolics (mg g^{-1} FW)	Total flavonoids (mg g^{-1} FW)	Antioxidant capacity (%DPPHsc)
Red seedless								
	Conventional	4 buds	23.3 b	0.545 b	42.8 a	2.10 b	0.0101 bcd	77.1 b
		6 buds	23.0 c	0.538 b	42.8 a	1.88 bc	0.0092 cde	15.4 d
		8 buds	23.1 bc	0.541 b	42.7 a	1.43 cd	0.0133 a	76.3 c
	Trellis	4 buds	22.9 bc	0.618 a	37.1 b	2.12 b	0.0116 abc	76.2 c
		6 buds	22.8 d	0.613 a	37.2 b	2.91 a	0.0124 ab	71.9 e
		8 buds	24.2 a	0.614 a	39.4 b	2.42 ab	0.0119 abc	78.7 a
White seedless								
	Conventional	4 buds	22.5 c	0.633 a	35.5 b	1.09 cd	0.0098 ab	72.9 d
		6 buds	24.3 a	0.629 a	38.6 ab	2.09 a	0.0089 c	76.9 ab
		8 buds	22.7 c	0.628 a	36.1 b	2.14 a	0.0107 a	74.2 cd
	Trellis	4 buds	22.2 d	0.579 b	38.3 ab	1.33 c	0.0086 c	77.3 a
		6 buds	23.2 b	0.583 b	39.8 a	1.01 d	0.0093 bc	75.3 bc
		8 buds	23.2 b	0.580 b	40.0 a	2.06 a	0.0099 ab	75.2 bc

Means within each column followed by the same letter are not different at $P \leq 0.01$ based on Tukey test.

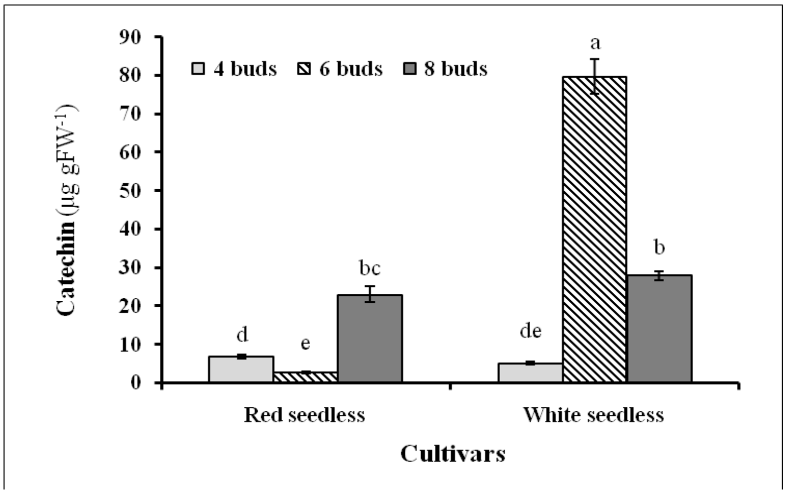

Fig 2. Effects of bud pruning on the catechin content of red and white seedless table grapes. The values are the means ± SE.

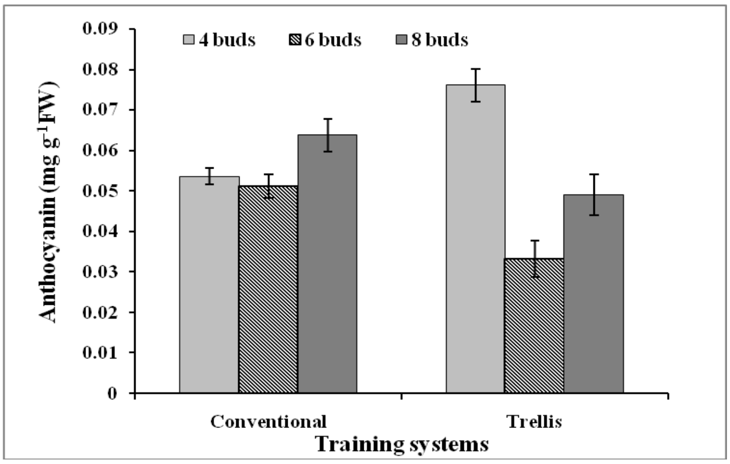

Fig 3. Effects of training systems and bud pruning on the total anthocyanin content of red seedless table grapes. The values are the means ± SE.

DPPH is one of the famous free radicals and is usually used for testing preliminary radical scavenging activity of a compound or plant extract. The free radical scavenging property may be one of the mechanisms by which this drug is effective in traditional medicine (Ghasemzadeh et al., 2010). Direct solar irradiance causes exposed fruits to overheat and reach temperatures +10 °C higher than the shaded fruits. These conditions expose fruits to an excess of light, temperature or an excess of excitation energy. Excess light is stressful to plants on a daily basis and could reduce the activity of chloroplast antioxidants while up-regulating the biosynthesis of flavonoids, even in the absence of UV irradiance. Thus, the activity of flavonoids may constitute a secondary antioxidant system that is activated as a consequence of the depletion of antioxidant enzyme activity (Agati et al., 2012). According to previous studies, a positive and significant relationship has been observed between total phenolics content, total flavonoids and antioxidant activities in plants (Ghasemzadeh et al., 2010). Polyphenols act as antioxidants through different mechanisms: (1) hydroxyl groups with π electrons of the phenyl can capture free radicals; (2) the generation of free radicals catalyzed by metals is diminished since they chelate metallic ions; (3) the cycle of generating new radicals is stopped through the donation of a proton from the phenolic compounds to the radicals and (4) polyphenols inhibit pro-oxidant enzymes that generate free radicals, such as lipoxygenases, cyclo-oxygenases and xanthine oxidase (Parr and Bolwell, 2000). In our study, it seems that different light intensities had a direct effect on antioxidant activities in plants with increasing total phenolics and total flavonoids and antioxidant content.

Total anthocyanin

The results showed that the bud pruning treatment had no significant effect on total anthocyanin content in conventional training system, whereas total anthocyanin content significantly affected by bud pruning in trellis system (Fig. 3). The highest total anthocyanin content (38.05 mg L^{-1}) was found in 4 buds pruning of trellis system. In most cases, a red seedless grape must exceed a minimum anthocyanin content to be accepted for sale, and growers may receive extra compensation for fruit with high anthocyanin content. Furthermore, grapes that are redder in color are more desirable to consumers for their appearance and potential antioxidant benefits. Berries with high anthocyanin content are considered to be of higher quality by buyers (Vinson et al., 2001). In addition to yield, fruit quality has also been shown to be affected by light penetration. Anthocyanin concentration (red pigment) in the fruit vitally depends on adequate light penetration (Toledo et al., 1993, and Strik and Poole, 1991). Onayemi et al. (2006) performed pruning experiments that showed an increase in total anthocyanin production and total flavonol concentration with an increase in light penetration. Light has been shown to be the most important environmental factor influencing anthocyanin biosynthesis in plants (Zhou and Singh, 2004), although in some species, such as *Vitis vinifera* cv. Shiraz anthocyanin accumulation appears not to be light-sensitive (Downey et al., 2004). Phytochromes are among the most extensively researched photoreceptors which sense light, and are known to be involved in anthocyanin biosynthesis (Zhou and Singh, 2004). In conclusion, this study showed that trellis training in combination with moderate bud loading had the higher nutrient quality -total phenolics content, flavonoids compounds and antioxidant capacity- than conventional system. In light of these results, commercial training of red and white seedless table grape cultivars on trellis system with moderate bud pruning can be considered for increasing of table grape quality and consumers' acceptance.

Materials and Methods

Plant materials

The experiment was conducted during the 2012 growing season on two commercial mature white and red seedless vineyards located in Takestan in Qazvin province (Table 1) in Iran. The region characterized by a mountain climate, with semi-hot and dry summers and cold winter, an average annual rainfall of 302 mm and average annual temperature is 13.0 °C. Two Iranian popular cultivars were selected assuming the vines age, vegetative and reproductive characteristics to be similar. The vines were trained on conventional (creeper) and trellis systems in a medium-textured soil. The vines and the soil were managed according to standard cultural practices. The vines were regularly drip-irrigated during the season, water was supplied based on evaporative demand; meteorological data were recorded throughout the study. Pruning treatments of vines were performed to 4, 6, and 8 buds per vine, retained on both canes and spurs, with three replications per treatment. Each replication consisted of 10 vines selected for their uniformity. Fruits harvested at ripe stage and then were selected for size and color uniformity. Blemished, damaged or diseased berries were discarded carefully. After preparation, fruits were transferred to the laboratory and some physiochemical parameters were determined.

Soluble solid content (SSC), Titratable acidity (TA) and SSC/TA

SSC (°Brix) was determined by a digital refractometer (CETI-Belgium) at 22 °C and expressed as a percentage for 15 berries juice for each treatment. TA was determined by titration of 30 mL filtrated juice with adding of 0.1 N NaOH solutions to the juice to reach the pH of 8.2. TA content was expressed as a percentage of tartaric acid.

Preparation of grape extracts

Grape tissue (3 g) was homogenized and extracted in 5 mL of methanol:acetic acid (85:15, v/v) according to Bakhshi and Arakawa (2006) with some modifications. The extract was filtered through Whatman No. 41 paper and then kept in refrigerator overnight. Aqueous part of the samples were centrifuged for 10 min at 7826 ×g and then stored at -20 °C until used for analysis of the total phenolics, total flavonoids, flavonoid compounds and antioxidant capacity.

Determination of total phenolics, total flavonoids, flavonoid compounds and antioxidant capacity

Total phenolics content was determined using the Folin-Ciocalteu method as described by Singleton et al. (1999) with a minor modification. 40 µl of methanolic extract was brought to a volume of 500 µl with distilled water in test tubes, followed by addition of 2.5 mL of 10% Folin–Ciocalteu reagent and allowed to stand for 5 min. Thereafter, 2 mL of 7.5% sodium carbonate solution was added. Each sample was allowed to stand for 90 min at room temperature in darkness and the absorbance was measured at 760 nm. The absorbance was read at 765 nm by an UV/Vis spectrophotometer model PG Instrument $^{+}$80 (Leicester, UK), and the total polyphenols concentration was calculated from a calibration curve, using gallic acid as standard (5–120 mg/L). Results are expressed as mg gallic acid $100g^{-1}$ fresh weight.

Total flavonoids content, were analyzed spectrophotometrically as described by Du et al. (2009) with some modifications. 150 µL of methanolic extracts were mixed with 1700 µL ethanol (30%), 75 µL $NaNO_2$ (5%), and 75 µL $AlCl_3$ (10%) were added. The extract samples were mixed and after 5 min, neutralized with 2 mL NaOH solution (1 M). The absorbance was measured at 506 nm and the quantification was carried out using a standard curve prepared from authentic catechin. Finally, the results are expressed as mg catechin equivalents (RE) per 100 g of sample. Flavonoids were determined using high-performance liquid chromatography (HPLC) as described by Bakhshi and Arakawa (2006). The methanolic extract of centrifuged samples was filtered through disposable 0.45 µm syringe filter. 50 µL of the filtered sample was injected into HPLC (Waters, 1525, Milford, CT, USA) equipped with a UV-visible detector (Waters Dual k Absorbance 2,487), a column (Waters Symmetry C18; 5 µm pore size; 4.6 mm × 150 mm; Waters, Dublin, Ireland). Samples and the column were held at 25°C, the eluent flow was at 1.0 mL min^{-1}, and the run time was 40 min. Flavonoids were identified by comparing their retention time with that of a corresponding standard and by spiking samples with the standard. Flavonoids concentration was calculated from the absorbance at 280 (for catechin) and 350 nm (for quercetin) and was expressed in µg mL^{-1}. Catechin standard was purchased from Sigma-Aldrich chemicals and quercetin was received from Extrasynthese, France. The antioxidant capacity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method according to the procedure of Du et al. (2009) with some modifications. Briefly, 50 µL of methanolic extracts were added to 950 mL of DPPH (6.25×10^{-5} M) radical and mixed by vortex and allowed to stand 30 min at room temperature in darkness. The absorbance of the samples were measured at 517 nm by UV/Vis spectrophotometer. The antioxidant activity was expressed as the percentage of decline of the absorbance, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH, which was scavenged (%DPPHsc), was calculated using:

$\%DPPHsc = [(A_{cont} - A_{samp}) \times 100]/ A_{cont}$

where A_{cont} is the absorbance of the control, and A_{samp} is the absorbance of the sample.

Total anthocyanin

Total anthocyanin content was determined only in red seedless grape. Spectrophotometric analysis of total anthocyanin content in savory stem was determined using of the pH differential method (Lee et al., 2005). Briefly, absorbance of each extracted sample was measured at 520 and 700 nm by two buffers at pH 1.0 and 4.5. Then absorbance was calculated using:

$A = [(A_{520}–A_{700})\ pH_{1.0} – (A_{520}–A_{700})\ pH_{4.5}]$

formula by the molar extinction coefficient of malvidin-3-glucoside. The total anthocyanin was calculated using:

Total anthocyanin (mg L^{-1}) = $(A\times MW \times DF \times 1000)/(\varepsilon \times 1)$

formula, where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorptive.

Statistical analysis

Two-factorial field experiment established according to randomized split-plot design with three replications. Each main plot included both training and pruning treatments with their various levels and replications. Data were analyzed as a combined experiment model by PROC ANOVA procedure by SAS software (Ver. 9.1 2002–2003, SAS Institute, Cary, NC, USA). Before analysis of variance, data were tested for normality and homoscedasticity using the Kolmogorov–Smirnov and Cochran tests, respectively. Variance analysis and Tukey's tests were performed at $P \leq 0.01$ to compare differences between means when F values were significant.

Conclusion

Training system and pruning severity could affect fruit quality. In the regions with high sunlight leaving long canes result in higher sugar content and antioxidant substances. Trellis system increased sunlight penetration, which in turn increased yield and fruit quality with higher phenolics and higher antioxidant capacity than conventional system. In the case of anthocyanin accumulation which is highly light-related procedure, sever pruning (4-buds) showed the best result. Overal, trellis training system could be recommended for the areas free of winter and early spring frost.

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