

## 5BB anacı ve Cabernet Sauvignon (*Vitis vinifera* L.) üzüm çeşidinin adventif kök oluşumu üzerine Melatonin ve IAA'nin etkileri

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### ÖZET

Triptofandan sentezlenen melatonin (MEL) ve indol asetik asit (IAA) iki *Vitis* türünün (5BB anacı ve Cabernet Sauvignon çeşidi) köklenmesi üzerine etkileri açısından denenmiştir. İki gözlü çelikler, bazal kısımları aşağıda belirtilen uygulamalar içine daldırıldıktan sonra iklim odasında yetiştirilmiştir: 0 (kontrol), 5.7, 11.4 ve 16.1 µM konsantrasyona sahip IAA solüsyonunda 5 saniye ve 0 (kontrol), 0.1, 0.5 ve 1.0 µM konsantrasyona sahip melatonin solüsyonlarında 10 dakika. Bulgular her iki türde köklenme, sürme ve sağlıklı bitki yüzdelerinin istatistiksel anlamda uygulamaların önemli etkisi altında olmadığını göstermiştir. Ancak artan IAA konsantrasyonlarının 5BB anacında köklenme yüzdesini de yükselttiği görülmüştür. Diğer yandan 0.1 ve 0.5 µM MEL uygulamaları da çeliklerde köklenmeyi teşvik etmiştir. Cabernet Sauvignon'da 11.4 µM IAA en yüksek yüzdeyi (%100) sağlamıştır. Melatonin köklenmede IAA'ya göre göreceli bir düşüşe neden olmuştur ve Melatoninin köklenme üzerindeki işlevi IAA'dan bağımsız gibi görünmektedir. Hormon uygulamaları 5BB'de çelik başına kök sayısı ve taze kök ağırlığı üzerinde önemli etkilere neden olmuştur. Cabernet Sauvignon'da hormonların etkisi, kök sayısı, kök uzunluğu, kök dağılım şekli ile yaş ve kuru ağırlıkları da dahil olmak üzere köklenme ilgili tüm özellikler üzerine etkili bulunmuştur. Sonuçlar asmada köklenme üzerine Melatoninin etkisini belirlemek için detaylı çalışmaların yapılması gerektiğini ve ucuz olması ve saf halde kolaylıkla elde edilebilmesi nedeniyle Melatoninin çoğaltmada kullanılabilme olasılığı olduğunu göstermektedir.

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## Effects of Melatonin and IAA on Adventitious Root Formation in Rootstock 5BB and cv. Cabernet Sauvignon (*Vitis vinifera* L.)

### ABSTRACT

Both derived from tryptophan, melatonin (MEL) and indole acetic acid (IAA) were tested for their influence on rooting in the cuttings of two *Vitis* species, the 5BB rootstock and the cultivar Cabernet Sauvignon. The 2-bud cuttings were grown in a growth chamber after the basal ends were dipped in the following treatments; for 5 seconds in IAA solutions at 0 (the control), 5.7, 11.4 and 16.1 µM concentrations, and for 10 minutes in the melatonin solutions at 0 (the control), 0.1, 0.5 and 1.0 µM. The results indicated that percentages of rooting, shooting and healthy plants in both species were not statistically significant under the influence of the treatments. However, increasing concentrations of IAA resulted in increasing rooting percentages in 5BB rootstock. On the other hand, 0.1 and 0.5 µM MEL also induced rooting. In the cuttings of Cabernet Sauvignon, 11.4 µM IAA provided the highest percentage (100%). Melatonin caused a relative decrease in rooting compared to the IAA. The action of melatonin on rooting seems to be independent of IAA. Hormone treatments caused significant differences in the

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root number per cutting and fresh root weight in 5BB. Effects of the hormones were important on all the rooting features, including root number, root length, root distribution pattern, and weight of fresh and dry roots in Cabernet Sauvignon. Results indicate that extensive research is needed to extrapolate the influence of melatonin on rooting in grapevine with a possibility in practical use since it is inexpensive and easy to obtain in pure form.

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## INTRODUCTION

In woody perennial horticultural species, propagation with cuttings destined to be used in direct planting or in grafting purposes is almost compulsory to ensure the perpetuation of same genetic material over generations. In grapevine propagation, different rootstocks with varying degree of rooting ability make it necessary to treat the cuttings with auxins to increase root formation.

Using plant growth regulators on promoting cuttings to form adventitious roots has been a practice for a very long time. Among them, auxins are the one that has acquired the name root-promoting hormones. These are signals that not only affect root formation but also involve in variety of processes during plant growth and development (Woodward and Bartel, 2005). With the advent of new information regarding phytohormones and signal molecules, it is clear now that these compounds interact with each other during root formation, a process in which auxin is the major factor (Lavenus et al., 2013). In a coordinated manner, auxin acts with cytokinins, gibberellins, ethylene, abscisic acid, jasmonate and strigolactones, all of which have their own specific biosynthesis and signaling pathways. The evidence clearly indicate that root formation and growth depend on communication among these compounds (Pacifci et al., 2015).

Since the discovery in plants in 1995 (Dubbels et al., 1995; Hattori et al., 1995), melatonin (MEL), which was long thought a molecule found only in vertebrates, has drawn some attention regarding its role in regulating a plant's physiology. Particular attention was given since melatonin is an indoleamine compound, as auxins are, and both are derived from tryptophan in tryptophan-dependent IAA biosynthetic pathway. Similar molecular structure of these two molecules (IAA and MEL) led scientists to think that melatonin might have the comparable or similar stimulating effect as IAA under certain conditions (Arnao, 2014).

Considering that auxins (IAA, IBA, and NAA) are known for their inducing effects on adventitious root formation, a question regarding if MEL might have a matching effect has risen. Studies on lupin and barley (Hernández-Ruiz et al., 2004, 2005; Hernández-Ruiz

and Arnao, 2008) showed that MEL, depending on the concentration applied, had an influence on roots similar to IAA.

Melatonin in grapevine has been first identified by Iriti et al. (2006) in some *Vitis vinifera* L. cultivars. Other studies on grape tissues (Stege et al., 2010; Boccalandro et al., 2011; Vitalini et al., 2011a, 2011b, 2013) have followed. Meng et al. (2014) showed that treating roots of 1-year old Riesling canes with melatonin helped better growth by stimulating water-stress tolerance. To this date, best to our knowledge, an experiment regarding determination of effects of melatonin applied during cutting preparation on root formation and growth was not performed. In studies by Sarropoulou et al. (2012a, 2012b) it was stated that MEL increased rooting of some sweet cherry rootstock. Pioneering this work, a study was designed to see if MEL promotes adventitious root formation as IAA does, in an American rootstock and a *Vitis vinifera* L. cultivar.

## MATERIAL and METHODS

Cuttings prepared from the American rootstock 5BB, and the *Vitis vinifera* L. cv. Cabernet Sauvignon (CS), were obtained from The Viticulture Research Institute, Tekirdag, Turkey. These were chosen for their easy to root aspects. Cuttings were shortened to 2-bud sections, with the lower bud removed. They were cut straight at the bottom and 45° slanted at the top away from the bud. Plant growth regulator (PGR) treatments in which the basal ends (2 cm in depth) of the cuttings were dipped were carried out as follows: 5 second-dipping in IAA at 0 (the control), 5.7 µM (equivalent of 1000 ppm), 11.4 µM (eq. of 2000 ppm) and 16.1 µM (eq. of 4000 ppm) concentrations, and 10 minute-dipping in the melatonin (Sigma M5250) solutions at 0 (the control), 0.1, 0.5 and 1.0 µM. The concentrations of IAA were selected on previous studies on grapevine propagation. Those of MEL were selected from the studies of Arnao and Hernández-Ruiz (2007) on *Lupinus albus* L., Sarropoulou et al. (2012a, b) on sweet cherry rootstocks and Sarrou et al. (2014) on pomegranate. Treated cuttings were directly planted in plastic containers (20 x 10 x 8 cm dimensions) that contained a growing medium of 2: 1 (peat moss:perlite). They were kept at 24±1°C at a 16-

8 hours day/night cycle for 8 weeks or until they stopped growing and showed signs of shoot tip drying. During these weeks, no inflorescences could develop. The maintenance was done by just watering as the cuttings needed.

At the end of the experiment following characteristics were evaluated for both rooting and shooting aspects (Uzunoglu and Gokbayrak, 2018) percentages (%) of rooting and shooting along with healthy plant ratio which was determined by assessing the healthy development of root and shoots on the same vine, root number per vine, a longest root length (cm), weights of fresh and dry roots and ash ratio (%). In addition, distribution pattern of the root emerged was also assessed as the place of the roots developed on the quadrant plane at the bottom of the vine by denoting 0-4 scale (0 being no roots emerged, 4 being roots emerged from all quadrants).

#### Statistical analysis

The study was conducted as a completely random trial design with three replicates (30 cuttings in total) per treatment. A total of 480 cuttings were evaluated. Data were statistically analyzed using Minitab statistics software (Release 16 for Windows, Minitab Inc.). Significant differences between the means were tested with Tukey test.

## RESULTS

The effects of two indolamine compounds, IAA and MEL, on root and shoot development of 5BB and CS cuttings were evaluated in a growth chamber. The results indicate that percentages of rooting, shooting and healthy plants in 5BB rootstock were not statistically significant under the influence of the treatments (Figure 1A). However, it was obvious that increasing concentrations of IAA resulted in increasing rooting percent in 5BB rootstock. Interestingly, cuttings of MEL control (in water only for 10 min) provided a complete and total rooting. However, 5 sec. dipping (i.e., IAA control) resulted in loss of 6 cuttings out of 100, indicating the inherent rooting capacity of 5BB. It was also intriguing to see that 0.1 and 0.5  $\mu\text{M}$  MEL also gave plants with 100% roots. However, the highest concentration of MEL applications caused a decrease in rooting percentage (80%). Variations in the shooting and healthy plants stood out more compared to the rooting. With IAA applications, shooting percent showed undulations, changing from 86.7% (5.7  $\mu\text{M}$ ) to 100 (11.4  $\mu\text{M}$ ) and later 96.7% (16.1  $\mu\text{M}$ ). MEL-applied 5BB cuttings developed fewer shoots.

On the CS cuttings, root and shoot growth were evaluated in Figure 1B. The percentages of rooting, shooting and healthy plants were not affected by the IAA and MEL treatments. 11.4  $\mu\text{M}$  IAA provided the highest ratio (100%) in all counts. The 1000 ppm IAA

and 4000 ppm IAA applications gave the next best ratio in rooting, (96.7%). Shooting was supported by all the treatments at 100% level, except for the 0.5  $\mu\text{M}$  MEL and the MEL control. Healthy plant percentage took a downfall in all the applications, especially being the worst with MEL control. Melatonin caused a dramatic decrease in rooting compared to the IAA but applying 0.5 or 1.0  $\mu\text{M}$  melatonin still boosted rooting compared to 5 second dipping (IAA control) at least 3%.

Root aspects of the 5BB cuttings after 8 weeks of growth were also presented in Table 1. Statistically significant effect of IAA (16.1  $\mu\text{M}$ ) on root number was observed (19.93 roots per cutting). The number of roots in the rest of the treatments were between 12-14.5 roots. However, 0.1 and 0.5  $\mu\text{M}$  MEL resulted in the longest root (10,51 and 9.70 cm, respectively). Longest root length, root distribution pattern and dry root weight, on the other hand, were not affected. Fresh root weight was greatly influenced by the applications, being the heaviest with 0.5  $\mu\text{M}$  MEL (6.27 g) and the lightest with 1.0  $\mu\text{M}$  MEL (3.13 g).

## DISCUSSION

The studies involving adventitious root formation in *Lupinus albus* L. (Arnao and Hernández-Ruiz, 2007) and *Arabidopsis* (Koyama et al., 2013) state that IAA and melatonin might operate in the same direction. Even though adventitious root formation in another woody horticultural species, *Punica granatum* (Sarrou et al., 2014) and various sweet cherry rootstocks (Sarropoulou et al. 2012b) was shown to increase 1.7 times and 3,8 times, respectively, compared to the control plants, in this study the increase was genotype dependent and not as much. For instance, 5BB cuttings responded positively to the all treatments, except for 1.0  $\mu\text{M}$  MEL. Rooting in cv. CS cuttings without Melatonin, on the other hand, showed a decrease around 30%, compared to the 11.4  $\mu\text{M}$  IAA treatment which gave 100% rooting. Chen et al. (2009) stated the stimulatory effect of 0.1  $\mu\text{M}$  Melatonin on rooting performance of two days old *Brassica juncea* seedlings, indicating that age of the material could play a role.

Arnao and Hernández-Ruiz (2007) stated that when Melatonin used with IAA, it promoted rooting. Sarrou et al. (2014) also presented data supporting influence of combining IAA and Melatonin on boosting rooting in pomegranate cuttings. In the present study, combination of the two hormones was not tested but singly applied melatonin supported adventitious root formation to some extent, depending on the concentration and the genotype of *Vitis* sp. Sarropoulou et al. (2012b) reported in their study on *in vitro* rooting of various sweet cherry rootstocks that rooting percentage significantly changed with Melatonin concentration and that both 0.05 and 1.0

$\mu\text{M}$  Melatonin treatments resulted in around 35% rooting. In this study where a growth chamber was utilized, 1.0  $\mu\text{M}$  Melatonin better supported rooting in CS than in 5BB, indicating that the response was genotype specific. Arnao and Hernández-Ruiz (2018) reported that the inhibitory effect of Melatonin on root growth surfaces when the concentration is above 10  $\mu\text{M}$ . In the current study, this negative effect appeared at considerably low concentrations, such

that 1.0  $\mu\text{M}$  in 5 BB and 0.1  $\mu\text{M}$  in Cabernet Sauvignon cuttings. This might have been the result of the growth conditions and the state of the plant material. IAA and melatonin maintained a good shooting performance in the cuttings of both 5BB and cv. CS. However, the reaction of CS cv was contingent on the concentration. In contrast to our findings, shooting in pomegranate cuttings treated with single concentration of Melatonin (Sarrou et al., 2014a) was

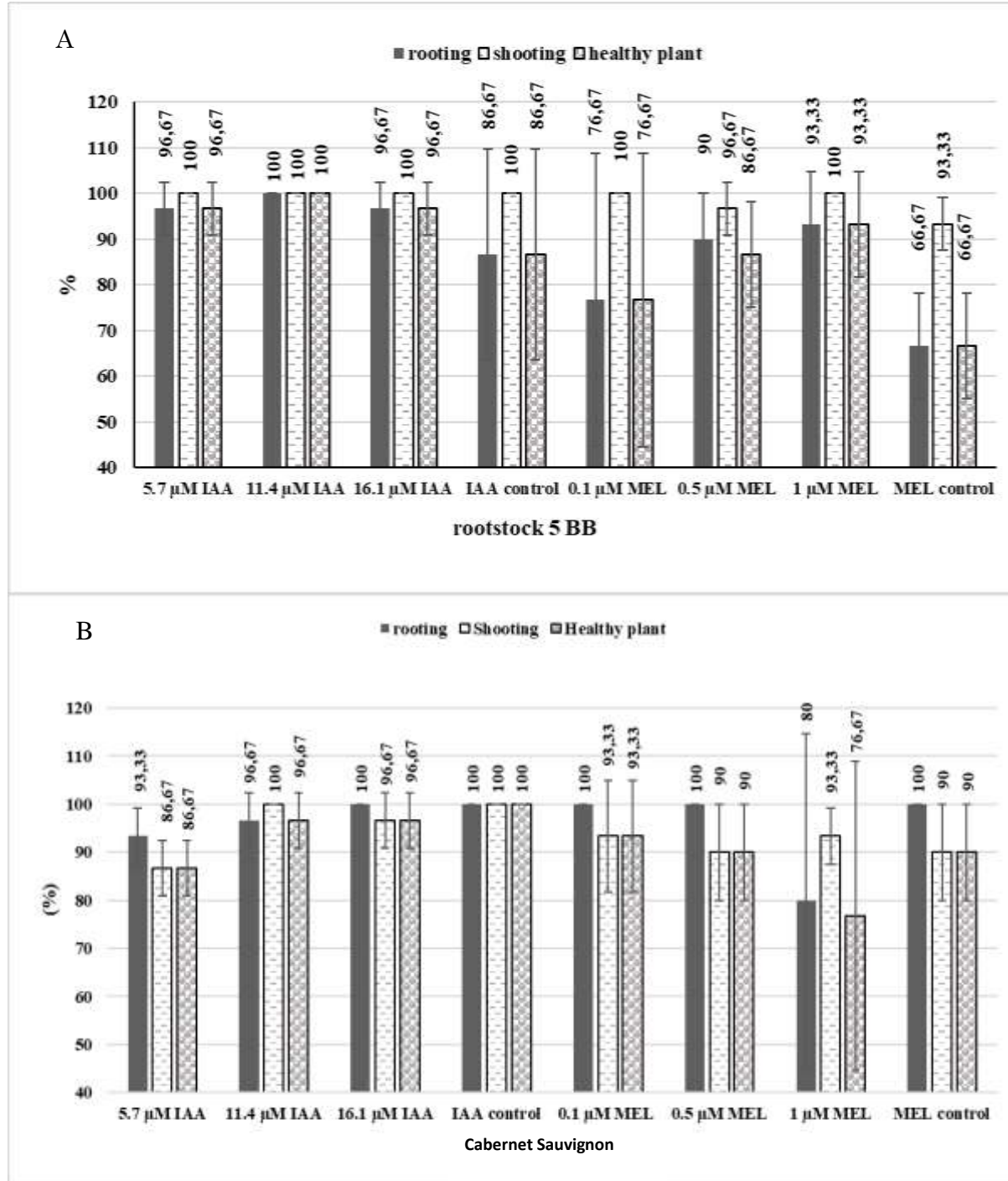


Figure 1. Effects of different concentrations of IAA and melatonin on cuttings of 5BB (A) and Cabernet Sauvignon (B) after eight weeks of growth in a growth chamber (vertical bars represent standard deviation)

Şekil 1. Farklı konsantrasyonlardaki IAA ve melatoninin büyüme odasında 8 haftalık büyüme sonrasında 5BB (A) ve Cabernet Sauvignon (B) üzerindeki etkileri (dikey çubuklar standard sapmayı göstermektedir)

Table 1. Root characteristics observed on rootstock 5BB cuttings treated with various concentrations of IAA and melatonin (Mean ± Standard deviation)

Çizelge 1. Farklı konsantrasyonlarda IAA ve melatonin ile muamele edilen 5BB çeliklerinde gözlenen kök özellikleri (Ortalama ± Standard sapma)

PGR treatments (BBD uygulamaları)	Root number (n) (Kök sayısı (adet))	Longest root length (cm) (En uzun kök uzunluğu (cm))	Root dist. pattern (0-4 scale) (Kök dağ. şekli (0-4 skala))	Fresh root weight (g) (Taze kök ağırlığı (g))	Dry root weight (g) (Kuru kök ağırlığı (g))
5.7 µM IAA	13.03± 1.04 b*	7.75± 0.51	3.33± 0.28	5.57± 0.79 ab	0.69± 0.11
11.4 µM IAA	14.53± 1.92 b	7.68± 0.88	3.30± 0.30	3.75± 0.18 bc	0.46± 0.05
16.1 µM IAA	19.93± 2.15 a	7.74± 0.74	3.93± 0.12	5.50± 0.92 ab	0.66± 0.05
Control (0 µM IAA)	13.77± 0.75 b	8.01± 0.27	3.47± 0.42	5.64± 0.46 ab	0.71± 0.10
0.1 µM MEL	13.43± 0.92 b	10.51± 1.09	3.37± 0.15	3.75± 0.60 bc	0.69± 0.06
0.5 µM MEL	14.43± 1.30 b	9.70± 0.70	3.37± 0.32	6.27± 1.38 a	0.72± 0.13
1 µM MEL	12.33± 6.26 b	7.40± 5.04	2.67± 1.36	3.13± 2.35 c	0.50± 0.36
Control (0 µM MEL)	13.70± 3.41 b	8.60± 0.87	3.30± 0.00	4.28± 0.71 abc	0.59± 0.12

\*Means with different letters in the column indicate significant differences at  $p < 0.05$ .

Table 2. Root characteristics observed on *Vitis vinifera* L. cv. Cabernet Sauvignon cuttings treated with various concentrations of IAA and melatonin (Mean ± Standard deviation)

Çizelge 2. Farklı konsantrasyonlarda IAA ve melatonin ile muamele edilen *Vitis vinifera* L. cv. Cabernet Sauvignon çeliklerinde gözlenen kök özellikleri (Ortalama ± Standard sapma)

PGR treatments (BBD uygulamaları)	Root number (n) (Kök sayısı (adet))	Longest root length (cm) (En uzun kök uzunluğu (cm))	Root dist. pattern (0-4 scale) (Kök dağ. şekli (0-4 skala))	Fresh root weight (g) (Taze kök ağırlığı (g))	Dry root weight (g) (Kuru kök ağırlığı (g))
5.7 µM IAA	29.17± 7.30 a*	7.70± 0.17a	3.40± 0.17 a	2.69± 0.93 bc	0.42± 0.10 ab
11.4 µM IAA	28.40± 2.60 ab	7.28± 0.19 a	3.60± 0.30 a	4.85± 1.66 a	0.57± 0.07 a
16.1 µM IAA	32.23± 4.24 a	7.29± 0.69 a	3.40± 0.44 a	4.09± 0.07 ab	0.56± 0.11 a
Control (0 µM IAA)	22.73± 6.81 abc	6.64± 0.79 a	3.13± 0.64 ab	3.00 ± 0.65 bc	0.40± 0.02 ab
0.1 µM MEL	14.03±10.51 cd	3.93± 2.50 b	2.17± 1.21 bc	1.37± 0.88 cd	0.18± 0.16 c
0.5 µM MEL	17.20± 2.52 bcd	5.59± 0.31 ab	3.13± 0.12 ab	1.93± 0.67 cd	0.27± 0.09 bc
1 µM MEL	14.87± 9.75 cd	5.57± 2.98 ab	2.90± 1.31 abc	1.80 ± 1.86cd	0.27 ± 0.20 bc
Control (0 µM MEL)	10.30± 1.39 d	3.43± 0.60 b	1.70± 0.17 c	0.82± 0.34 d	0.17± 0.04 c

\*Means with different letters in the column indicate significant differences at  $p < 0.05$ .

less than the control group, which might show that Melatonin reacts differently in different species.

Root growth aspects of the CS cuttings were under the influence of the hormones (Table 2). Root number was considerably high in the cuttings treated with IAA. However, the differences in the longest root length was not as distinct among the applications. IAA-treated cuttings tended to develop roots from all quadrants with high fresh and dry weights.

Mean number of roots in the cuttings used in the experiment were influenced by the PGR's, in agreement with the study by Sarropoulou et al. (2012b), which revealed that cherry rootstocks produced the highest number of roots at 0.5 and 1.0 µM, following the control. In the cherry rootstock, PHL-C, Sarropoulou et al. (2012a) found the decreasing effect of Melatonin on root number. In this study, 5BB cuttings significantly developed more roots with IAA than with Melatonin. However, with cv. CS, this difference was less prominent, spiking at 16.1 µM IAA and being at similar levels in the rest of

the applications. Effects of MEL on the length of the longest root was evident in 0.5 and 1.0 µM MEL. Even though Sarrou et al. (2014) presented the mean length of roots, they still reported the supportive influence of 0.1 and 1.0 µM MEL treatments. Sarrou et al. (2014) also showed that Melatonin caused a prominent increase in the length of the roots formed on the pomegranate cuttings. Hernández-Ruiz et al. (2005) on the other hand, showed that in monocot species IAA and MEL decreased root length.

Fresh weight of roots was generally low in 5BB and cv. Cabernet Sauvignon with melatonin, however 0.5 µM MEL caused a peak in 5BB. Sarropoulou et al. (2012a) stated that low MEL concentrations (0.05-1.0 µM) increased fresh root weight of PHL-C rootstock. The same tendency was also found with Gisela 6 and MxM 60, but not with CAB-6 P. These contradictory results might be due to genotype differences and sensitivities. The scientists stipulated that the increase could be from the volume increase in old cells and/or cell extension in new cells. Since dry weight did not increase in a parallel way to the fresh weight

it led us to think that the increase was due to a volume increase by accumulating more water in the cells.

## CONCLUSION

Results indicated that effects of Melatonin on adventitious root formation in the 5BB and Cabernet Sauvignon cv. need more extensive studies. Its effect is not the same or as much extent of IAA and depend on its concentration. Both genotypes certainly had a better response to IAA applications. Further studies involving the underlying mechanism which Melatonin might exert on root formation would enable us to find out more about the crosstalk among signal molecules. There might be a possibility of using Melatonin in practical applications since it is inexpensive and easy to obtain in pure form.

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## Statement of Conflict of Interest

Authors have declared no conflict of interest.

## Author's Contributions

The contribution of the authors is equal.

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