



Antioxidant Procedure Improve Olive Cuttings Rooting During The Cool Season



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COLD temperature during winter season consider an environmental constrains for root and shoot development of olive cuttings. This experiment was carried out during two seasons (2018/19 and 2019/20) to study the rooting ability of 'Coratina', 'Picual' and 'Manzanillo' olive cultivars in addition to study the effect of supplementary intermittent application of IBA and antioxidant (1:1 ascorbic and citric acid) at 100, 200, 300ppm three times after cuttings planting on their rooting behavior during cold winter. The results showed that, 'Coratina' olive cultivar recorded the highest significant rooting percent success (75.86 & 74.73%) and root number compared to 'Picual' and 'Manzanillo' cultivar. While, 'Manzanillo' cultivar recorded the lowest rooting percent, root number and root length. Antioxidant at 300ppm recorded the highest rooting percent 74.44 & 73.78%, root number and root length. Concerning the interaction effect, the highest significant rooting percent 94.67 & 96.00% and root number was achieved by 'Coratina' olive cultivar treated with 300 ppm antioxidant. While, the highest root length recorded by 'Coratina' cultivar with 100ppm IBA and the highest leaves number recorded by 'Manzanillo' cultivar under 100 ppm antioxidant. It can be concluded that, 'Coratina' olive cultivar was more suitable for winter season propagation. Also, supplementary application of 300ppm antioxidant was more effective in olive cuttings propagating, while 300ppm IBA had negative effects.

Keywords: Ascorbic, Citric, IBA, Olea europaea, Propagation, Intermittent, Tunnel.

Introduction

Olive tree (*Olea europaea* L.) consider one of the most important economic resources and diet for the Mediterranean region (Leone et al., 2015), which produce 98% of the olive oil production worldwide (IOC, 2019). Olive oil consumption and demand is increasing due to balanced fatty acid composition and antioxidant properties (IOC, 2019). Also, olive tree is a widely distributed in arid areas for ramified root system and special leaf structure (Tanasijeveca et al., 2014).

High quality transplants are necessary to success in fruit production. Vegetative propagation of olive via rooting leafy cuttings is widely used (Hartmann et al., 2002 and Fabbri et al., 2004), which considered easy, inexpensive, fast and appropriate for mass plant propagation. The greatest effect on rooting achieved by Auxins (Eid et al., 2018). IBA (indole butyric acid) is the most auxin used for rooting of olive cuttings (Hussein et al., 2017 and Shiri et al., 2019), therefore, the results found vary a lot according to the dosage, the cultivar, the period of rooting, the substrate and the type of cutting among other factors.

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Antioxidative substances like ascorbic acid (Patel *et al.*, 2020) and citric acid (Moola & Kumari, 2020 and Soonthornkalump *et al.*, 2020) are often reported in micropropagation, but it is still very rare in cuttings rooting. Ascorbic acid is an important component of antioxidant system in plants, which responsible for regulate many physiological activities such as H₂O₂ scavenging (Hasanuzzaman *et al.*, 2020), cell elongation (Paciolla *et al.*, 2019) and cell multiplication (Gallie, 2013, Lisko *et al.*, 2014 and Ellya Kka, 2017). Also, citric acid acts as a chelating agent, acidulant and PPO activity inhibitor (Rojas-Graü *et al.*, 2008, Hithamani *et al.*, 2018 and Zhou *et al.*, 2020). Ascorbic acid improved rooting percentage in *Stewartia pseudocamellia* cuttings before IBA (100mgL⁻¹) treatment (Struve and Lagrimini, 1999). More recently, El-Sharony *et al.* (2018) reported an increase in guava cuttings success after soaking in mixture of 5% citric acid + 5% ascorbic acid and treated with 3000ppm IBA compared to IBA treatment alone. Moreover, Radhi and Hussein (2020) revealed that Ascorbic acid alone induced rooting of *Acacia leprosa* when supplied individually rather than in combination with IBA.

Unfortunately, economically important olive cultivars that show intermediate or even easy rooting capacities and shoot growth had a low rooting success and shoot growth during winter nursling production (Fabbri *et al.*, 2004). During cool seasons endogenous hormones rate was decreased due to the decrease in metabolic activities of the trees so, higher concentrations of hormones are required (Bartolini *et al.*, 1986). In some Mediterranean country like Egypt, during cool season minimum temperature arranged from 19.1C° in September till 7C° in January (CDO, 2021) which unsuitable for olive growth. Propagation of olive cuttings under plastic tunnel is an ancient system, but nowadays for cold winter temperature, high price of electricity and irrigation water it was more suitable than fog irrigation system (mist). While, low cuttings rooting percent during winter cool season will increase propagation costs and decrease nursery production. So, the aim of this study was determine the rooting ability of between some olive cultivars in addition to improving olive cutting propagation procedure during cool season through supplementary application of IBA and antioxidants (Ascorbic and citric acid).

Materials and Methods

Experimental conditions

This study was carried out during two seasons
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2018/19 and 2019/20 on three olive cultivars namely i.e., 'Picual', 'Manzanillo' and 'Coratina'. Forty five cuttings of each cultivars were prepared in four nodes with 4 terminal leaves and 15cm length taken from one-year-old branches during September each year. All cuttings base dipped in IBA at 4000ppm for 20 seconds immediately before planting. After that, the cuttings treated with one of the following treatment i.e. treating the planted cuttings base through additional application of IBA at 100, 200 and 300ppm or mixture of antioxidant (1:1 of citric and ascorbic acid) at 100, 200 and 300ppm to the soil with approximately 300ml/ solution for each treatment. The soil Treated three times, each one every three days intervals started at planting day. The treatments added at night after mist stopped from 5.00 PM till 7.00 AM as modified and intermittent slow soaked treatments for 14 hours. The cuttings planted in sand : peat moss (4:1) under mist irrigation system which sprayed water 15 seconds every 15 minutes from 7.00am to 17.00pm for one month only. After that, the cuttings during cool season covered with polyethylene as a tunnel till the end of march and irrigated once a month. Cuttings rooting percent, roots number, roots length and new leaves number were recorded at the first of March.

Statistical analysis

This experiment was arranged in a randomized complete block design with two factors (olive cultivar X treatments) and subjected to variance analysis (Snedecor and Cochran, 1980). The means of the treatments were compared by least significant difference (Duncan, 1955) at 0.05 significance level. The treatments were factor A and the cultivars were factor B.

Results and Discussion

Cuttings rooting percent

Table 1 showed the effect of supplemental application of IBA and antioxidant on rooting percent of olive cuttings during cold season. It was cleared that, 'Coratina' olive cultivar recorded the highest significant rooting percent followed by 'Picual' while 'Manzanillo' cultivar recorded the significant lowest rooting percent. For the effect of supplementary application of IBA and antioxidant, it was cleared that, 300ppm followed by 200ppm antioxidant recorded the significant highest rooting percent. while the significant lowest rooting percent recorded by antioxidant at 100ppm and IBA at 300ppm. For the interaction effect, antioxidant at 300ppm

gave the highest value with all olive cultivars. Moreover, the significant highest values were recorded by 'Coratina' olive cultivars under 300ppm antioxidant.

Root number

Table 2 showed the effect of supplementary application of IBA and antioxidant on rooting numbers of olive cuttings during cool season. It was cleared that, 'Coratina' olive cultivar recorded the significant highest root numbers compared to 'Picual' and 'Manzanillo' cultivars. For the effect of supplementary application of IBA and antioxidant, it was cleared that, 300ppm antioxidant followed by 100ppm IBA gave significant highest root numbers compared to the other treatments, while the lowest root numbers recorded by IBA at 300ppm and antioxidant at 100ppm. For the interaction effect, antioxidant at 300ppm gave the highest value with 'Picual' and 'Manzanillo' olive cultivars while 100ppm IBA gave the significant highest root numbers for 'Coratina' cultivar. The significant highest root number recorded by 'Coratina' olive cultivars treated with 300ppm antioxidant, while the lowest value recorded by 'Manzanillo' olive cultivar under 100ppm antioxidant.

Root length

Table) showed the effect of supplementary application of IBA and antioxidant on root length of olive cuttings during cool season. It was cleared that 'Coratina' and 'Picual' cultivar recorded the significant highest root length compared to 'Manzanillo' cultivars. For the effect of supplemental application of IBA and antioxidant, it was cleared that, 300ppm antioxidant gave the significant highest root length compared to the other treatments. For the interaction effect, antioxidant at 300ppm gave the significant highest value with 'Coratina' and 'Manzanillo' olive cultivars while 300ppm IBA gave the best results for 'Picual' cultivar. While, the lowest ones was recorded by 'Coratina' cultivar under 300ppm IBA

Leaves number

Table 4 showed the effect of supplementary application of IBA and antioxidant on leaves number of olive cuttings during cool season. It was cleared that, 'Manzanillo' olive cultivar recorded the significant highest leaf number compared to 'Coratina' cultivar in both seasons. For the effect of supplemental application of IBA and oxidant , it was cleared that, 300 and 200ppm antioxidant

TABLE 1. Effect of supplementary application of IBA and antioxidant on rooting percent of three olive cultivars during cool season.

Treatments	Cultivars			Mean
	First season 2018 / 2019			
	Picual	Manzanillo	Coratina	
100ppm IBA	62.33 g	37.67 l	83.33 c	61.11 D
200ppm IBA	52.00 i	31.00 m	87.67 b	56.89 E
300ppm IBA	72.67 f	32.33 m	57.00 h	54.00 F
100ppm Antioxidant	61.33 g	23.00 n	47.67 j	44.00 G
200ppm Antioxidant	74.67 ef	44.67 k	82.33 c	67.22 B
300ppm Antioxidant	76.00 de	52.67 i	94.67 a	74.44 A
Control	61.33g	53.00 i	78.33 d	64.22 C
Mean	65.76 B	39.19 C	75.86 A	
	Second season 2019 / 2020			
100ppm IBA	61.67 g	37.33 l	81.67 c	60.22 C
200ppm IBA	54.00 i	30.00 m	88.00 b	57.33 D
300ppm IBA	72.00 e	27.00 n	56.67 h	51.89 E
100ppm Antioxidant	60.33 g	21.13 o	48.13 jk	43.20 F
200ppm Antioxidant	74.33 de	46.67 k	79.33 c	66.78 B
300ppm Antioxidant	76.00 d	49.33 j	96.00 a	73.78 A
Control	64.33 f	45.67 k	73.33 e	61.11 C
Mean	66.10 B	36.73 C	74.73 A	

Means followed by a different letters in the same column are significantly different at 0.05 level

TABLE 2. Effect of supplementary application of IBA and antioxidant on root number of three olive cultivars during cool season

Treatments	Cultivars			Mean
	First season 2018 / 2019			
	Picual	Manzanillo	Coratina	
100ppm IBA	4.367 jk	6.200 efgh	15.50 a	8.689 B
200ppm IBA	5.433 ghij	6.900 de	6.833 de	6.389 D
300ppm IBA	6.333 efg	4.467 jk	3.467 k	4.756 E
100ppm Antioxidant	4.833 ij	5.067 hij	4.833 ij	4.911 E
200ppm Antioxidant	8.667 c	7.833 cd	6.667 def	7.722 C
300ppm Antioxidant	12.33 b	9.000 c	12.33 b	11.22 A
Control	5.533 fghij	6.000 efghi	4.667 jk	5.400 E
Mean	6.786 B	6.495 B	7.757 A	
	Second season 2019 / 2020			
100ppm IBA	4.100 k	6.200 fgh	16.80 a	9.033 B
200ppm IBA	4.933 ijk	6.833 def	7.467 cde	6.411 CD
300ppm IBA	5.533 ghi	4.600 jk	2.733 l	4.289 F
100ppm Antioxidant	5.267 ij	7.267 cde	2.867 l	5.133 E
200ppm Antioxidant	7.800 c	7.267 cde	5.400 hij	6.822 C
300ppm Antioxidant	11.03 b	7.667 cd	11.00 b	9.900 A
Control	6.667 ef	5.000 ij	6.333 fg	6.000 D
Mean	6.476 B	6.405 B	7.514 A	

Means followed by a different letters in the same column are significantly different at 0.05 level

TABLE 3. Effect of supplementary application of IBA and antioxidant on root length of three olive cultivars during cool season

Treatments	Cultivars			Mean
	First season 2018 / 2019			
	Picual	Manzanillo	Coratina	
100ppm IBA	7.100 def	6.333 fghi	7.500 cde	6.978 B
200ppm IBA	6.000 ghij	5.233 j	7.000 defg	6.078 C
300ppm IBA	8.833 ab	5.833 hij	5.500 ij	6.722 B
100ppm Antioxidant	6.667 efgh	5.100 j	6.500 efghi	6.089 C
200ppm Antioxidant	6.967 defg	5.467 ij	7.833 bcd	6.756 B
300ppm Antioxidant	8.167 bc	7.933 bcd	9.500 a	8.533 A
Control	7.167 cdef	6.333 fghi	6.667 efgh	6.722 B
Mean	7.271 A	6.033 B	7.214 A	
	Second season 2019 / 2020			
100ppm IBA	6.867 bc	5.733 de	7.467 b	6.689 BC
200ppm IBA	6.200 cd	5.600 de	7.633 b	6.478 BC
300ppm IBA	8.833 a	5.100 de	4.933 e	6.289 C
100ppm Antioxidant	7.067 bc	5.467 de	7.100 bc	6.544 BC
200ppm Antioxidant	6.933 bc	6.200 cd	7.667 b	6.933 B
300ppm Antioxidant	7.500 b	7.667 b	9.333 a	8.167 A
Control	5.500 de	5.333 de	5.333 de	5.389 D
Mean	6.986 A	5.871 B	7.067 A	

Means followed by a different letters in the same column are significantly different at 0.05 level

beside 100ppm IBA gave significant highest leaves number, while the significant lowest roots number recorded by IBA at 300ppm and antioxidant at 100ppm. For the interaction effect, antioxidant at 300ppm gave the highest value with 'Coratina' and 'Manzanillo' olive cultivars, while 300ppm IBA gave the best results for 'Picual' cultivar, while the lowest ones was recorded by 'Coratina' cultivar under 100ppm antioxidant.

Discussion

The results indicated that, for the effect of cultivars, it is clear that under cool season 'Coratina' olive cultivar gave the highest rooting parameters, while 'Manzanillo' cultivar recorded the lowest values. In this regard Wiesman and Lavee, (1995a&b) found that, 'Manzanillo' and 'Picual' considered moderate to rooting cultivars. Concerning the effect of treatments, the results cleared that, antioxidant (Ascorbic and citric acid) have a great effect on rooting percent, root number, root length and leaves number of olive cuttings under this study, which improved rooting parameters under high concentration (300ppm) and decreased it under low concentration (100ppm). These results was agreement with Struve and Lagrimini, (1999) as they mentioned

that ascorbic acid improved rooting of *Stewartia pseudocamellia*. Also, El-Sharony et al. (2018) as they observed an increase in guava cutting success which treated with mixture of 5% citric acid + 5% ascorbic acid compared to cuttings treated with 3000ppm IBA alone. This results may be due to that, ascorbic acid acts within the meristems as a necessary factor for cell division (Gallie, 2013, Lisko et al., 2014 and Ellya Kka, 2017) and cell elongation (Paciolla et al., 2019). Moreover, ascorbate stimulate dividing of quiescent cells similar to auxin activity which known as a rooting hormone in suitable concentration (Kaviani, 2014). Furthermore, Ascorbic acid possess some physiological activity related to rooting such as increasing mitotic index of root quiescent center and apical meristem, increasing cellular dimensions in cell elongation zone, increasing root mitotic divisions, increasing secondary roots production through pericycle cells stimulation and divisions (Kaviani, 2014). Potters et al. (2002) observed that ascorbic acid included in regulate passing cell through cell cycle. In addition, citric acid consider chelating agent, polyphenol oxides inhibitor, Cu-chelating agent (Jiang et al., 1999, Moline et al., 1999 and Rojas-Graü et al., 2008). More recently, Radhi and Hussein (2020) found

TABLE 4. Effect of supplementary application of IBA and antioxidant on leaf number of three olive cultivars during cool season

Treatments	Cultivars			Mean
	First season 2018 / 2019			
	Picual	Manzanillo	Coratina	
100ppm IBA	8.333 hij	11.50 b	13.17 a	11.00 A
200ppm IBA	7.833 ij	10.83 bcd	10.83 bcd	9.833 B
300ppm IBA	9.833 ef	5.833 k	2.500 l	6.056 D
100ppm Antioxidant	7.500 j	13.27 a	1.967 l	7.578 C
200ppm Antioxidant	12.83 a	9.500 efg	10.17 cde	10.83 A
300ppm Antioxidant	11.50 b	9.167 fgh	12.50 a	11.06 A
Control	11.00 bc	10.00 def	8.667 ghi	9.889 B
Mean	9.833 A	10.01 A	8.543 B	
Second season 2019 / 2020				
100ppm IBA	8.667 d	11.40 bc	12.67 a	10.91 A
200ppm IBA	6.867 e	10.60 c	11.20 c	9.556 B
300ppm IBA	11.20 c	5.533 f	2.200 g	6.311 E
100ppm Antioxidant	6.600 e	12.53 a	1.933 g	7.022 D
200ppm Antioxidant	12.40 a	9.333 d	9.400 d	10.38 A
300ppm Antioxidant	11.33 c	8.633 d	12.33 ab	10.77 A
Control	6.000 ef	6.667 e	11.00 c	7.889 C
Mean	9.010 AB	9.243 A	8.676 B	

Means followed by a different letters in the same column are significantly different at 0.05 level

that Ascorbic acid ethe electronic conjugation system and inducing rooting response by inhibiting IAA- oxidase or GH3 enzyme activity which increase IAA level. With respect to IBA treatments, the results cleared that, increasing IBA concentration decreased rooting parameters and leaves number of the studied olive cultivars. With regard to the effect of concentration of IBA, raising IBA concentration from 3000ppm to 5000ppm decreased significantly cutting rooting percent from 60% to 20% beside decreasing root length and number of Coratina olive cuttings (Kurd *et al.*, 2010). The previous results were agreement with cornu (1973) who found that IBA had a broad ranges of root-stimulating activity, since 24.6 mM IBA was the best treatment compared to 98.6 mM IBA in *Pseudotsuga menziesii*. Also, little and too much auxin (IBA and NAA) decreased rooting (cornu, 1973). Moreover, the highest callus formation and rooting percent of black mulberry cuttings was in 3000 ppm dose application (68%) and the lowest one was in 4000 ppm dose application (17%) compared to the control (Kalyoncu *et al.*, 2009). Furthermore, 500 ppm IBA treatment gave best results compare to 1000ppm in *Tamarix aphylla* L. cuttings (AI Makhmari, 2016). Similarly, Owais (2010) found that 9000ppm IBA gave the highest rooting percent and root length compared to 12000ppm IBA in pomegranate cuttings. More recently, Muller *et al.* (2005) found that the application of 0.1 and 1 mM IBA increased rooting percent, but rooting completely inhibited under rate of 100 mM IBA.

For the time of IBA treatment, treated olive cuttings from 17.00pm till 07.00am permitted 14 h three time (total 42 intermittent hour) were similar to long soaked period. These results were similar to Inocent *et al.* (2018) as they found that increasing soaked time from 1h to 8h gradually decreased rooting percent of 'arbosana' from 66.7 to 14.6% and 'frantoio' olive cultivar from 33.3 to 10.4%.

Conclusion

During cool winter 'Coratina' olive cultivar followed by 'Picual' is more suitable for cuttings propagation than 'Manzanillo' cultivar. Also, 300ppm antioxidant is more effective than IBA as supplementary treatments for increasing rooting percent of olive cuttings during cold season.

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Conflict of interest

The authors declare no conflict of interest or personal relationships that could have appeared to affect the work reported in current study

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استخدام مضادات الأكسدة تحسن من تجذير عقل الزيتون خلال الجو البارد

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الحرارة المنخفضة خلال موسم الشتاء تعتبر من العوائق البيئية لتجذير و نمو عقل الزيتون. وقد أجريت هذه الدراسة خلال موسمين (١٩/٢٠١٨ - ٢٠/٢٠١٩) لدراسة تأثير استخدام الإضافات المنقطعة بعد زراعة العقل من كلا من إندول حامض البيوترريك ومضادات الأكسدة (١ حامض الأسكوربك : ١ حامض الستريك) بتركيزات ١٠٠، ٢٠٠، و ٣٠٠ جزء في المليون منفردة لكل مادة منهما على تجذير أصناف الزيتون كوراتينا، وبيكوال، ومنزانييلو خلال موسم الشتاء البارد. أشارت النتائج إلى أن عقل الزيتون صنف 'Coratina' سجلت أعلى نسب تجذير (٧٥,٨٦ & ٧٤,٧٣٪) وعدد جذور مقارنة بالصنفين الآخرين. بينما سجل الصنف منزانييلو أقل نسبة تجذير وعدد وطول للجذور. بالإشارة إلى استخدام مضادات الأكسدة أشارت النتائج إلى أن التركيز المرتفع ٣٠٠ جزء في المليون سجل أعلى درجة تجذير (٧٤,٤٤ & ٧٣,٧٨٪) وعدد الجذور (١١,٢٢ & ٩,٩) وطولها (٨,٥٣ & ٨,١٦ سم) مقارنة بالمعاملات الأخرى. وفيما يتعلق بالتأثير المتداخل بين المعاملات والأصناف فإن أعلى نسبة تجذير قد سجلت مع الصنف كوراتينا (٩٤,٦٧ & ٩٦,٠٠٪) كما سجل أعلى طول للجذور (٩,٥ & ٩,٣٣ سم) بالتداخل مع استخدام مضادات الأكسدة بتركيز ٣٠٠ جزء في المليون. بينما سجل أعلى طول للجذور مع الصنف كوراتينا باستخدام IBA بتركيز ١٠٠ جزء في المليون (١٥,٥ & ١٦,٨ سم)، كما سجل أعلى عدد للأوراق مع الصنف منزانييلو (١٣,١٧ & ١٢,٦٧) مع استخدام مضادات الأكسدة بتركيز ١٠٠ جزء في المليون. لذا يمكن التوصية بأن الصنف كوراتينا أكثر ملائمة للإكثار خلال الشتاء البارد. كذلك فإن المعاملات الإضافية المنقطعة من مضادات الأكسدة بتركيز ٣٠٠ جزء في المليون أكثر فاعلية في زيادة نسب التجذير، بينما IBA بتركيز ٣٠٠ جزء في المليون له تأثيرات سلبية.