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Root Growth of Arbequina Cuttings as Influenced by Organic and Inorganic Substrates under the Conditions of Al-Jouf (KSA)

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ABSTRACT

'Arbequina' olive cultivar which is the most grown for high quality olive oil in Spain, has the potential for being cultured in KSA. The aim of this study was to investigate the efficiency of inorganic and organic growing substrates on the root growth and the root morphology of Arbequina olive cuttings. One-year-old cuttings were dipped in IBA 4000 ppm before planting in 3 substrates: sand, perlite (inorganic) and peat moss (organic). The results showed that IBA (1H-indole-3-butyric acid) at 4000 ppm combined with perlite substrate were successful in promoting earlier root development and limited callus formation in the early period of rooting. Cuttings rooted more profusely on perlite substrate and developed significantly more and longer roots per cutting. Also best survival and easily acclimatization of plants in greenhouse was unregistered. On the contrary, peat moss developed a root system with high callus and proved to be less qualitative with less number and shorter roots per cutting, suggesting that peat moss was not a suitable environment for adventitious root formation of Arbequina cultivar. Good callus formation was also obtained with untreated control cuttings where late emergence was observed. Roots of cuttings necrosed and died by the end of growing period. Considering all studied aspects of root system, the physical properties of substrates should be carefully choose as rooting medium of olive cuttings.

Key words: *Olea europaea*, propagation, rooting substrate, root morphology, 1H-indole-3-butyric acid

INTRODUCTION

To increase the olive acreage in Kingdom of Saudi Arabia (KSA) during the past decade, the use of self-rooted planting stocks for the establishment of new orchards was more and more necessary. The economic method of vegetative clonal propagation is semi hardwood cuttings (Davies and Hartmann, 1988; Dvin *et al.*, 2011b) which is the most common system of obtaining new olive plants by asexual propagation (Fabbri *et al.*, 2004). But the low rooting quality of cuttings in some cultivars represent a limit factor (Epstein and Wiesman, 1987). Gerrakakis and Ozkaya, (2005), Isfendiyaroglu *et al.* (2009) and Awan *et al.* (2003) showed a significant variation in rooting ability of olive cuttings due to physical properties of substrate.

New olive (*Olea europaea* L.) orchards are being implanted in many semi arid areas as in Kingdom of Saudi Arabia, where water is the main limited factor. In arid conditions, Polverigiani *et al.* (2011) proposed that, plants produced roots of different functions and defined in woody plants two categories of roots: pioneer for the framework of the tree and fibrous roots which role is water and nutrient absorption. Root quantity and quality of olive cuttings in nursery are important to study because the root system developed, has the potential to draw water and mineral resources from the soil it explores and to provide necessary ingredients for optimal growth and development in the olive trees (Weissbein *et al*., 2006).

The key step in vegetative propagation is adventitious root formation which comprises three successive interdependent physiological phases: induction, initiation and expression (Gaspar *et al.,* 1992). Previous researches studies have reported that this process is related to physical properties of substrates, auxins and environmental conditions (Bir and Bilderback, 2003; Hartmann *et al.,* 1990). It is well known that different rooting substrate types and their properties are an important factor in determining the rooting habit of cuttings in greenhouse and rooting habit of the olive tree in field conditions (Loach, 1988). The physical properties of the rooting substrate can influence rooting and root quality, especially in more difficult-to-root olive cultivars (Loreti and Hartmann, 1964; Gerrakakis and Ozkaya, 2005) and a good water management is also crucial for success (Sebastiani and Tognetti, 2004; Dvin *et al*., 2011a). Ozenc and Ozenc (2007) signaled the importance of water content and aeration capacity of substrates and noted that inorganic materials were found more suitable for rooting; perlite is by far, the most used rooting substrate in olive propagation.

Hormones play also an important role in the rooting initiation (Loreti and Hartmann, 1964; Davies and Hartmann, 1988; Hartmann *et al*., 1997; Asl Moshtaghi and Shahsavar, 2011) and the effect of IBA (Indole Butyric Acid) at different concentrations on cutting root formation was reported in several olive cultivars (Ahmed *et al.,* 2002; Isfendiyaroglu and Ozeker, 2008; Turkoglu and Durmus, 2005).

The objective of this study was proposed to evaluate the effects of rooting substrate (organic and inorganic) and IBA treatments (0 and 4000 ppm) on root quality and on the earliness of the root system of Arbequina cuttings grown in Al-Jouf, Kingdom of Saudi Arabia (KSA). This study has also focused on specific aspects of root system development in the substrates tested. Organic material such as peat moss was used for its high level in organic matter and nutrient element content while sand and perlite with inorganic origin were preferred for their porosity.

MATERIALS AND METHODS

Study site: The experiment was conducted during the period from mi-October 2010 to January-February 2011 at the olive research unit located in Camel and Range research center of Al-Jouf, KSA (altitude of 684 m above sea level, latitude 29,8°N and longitude 40,1°E). The mean temperature (°C) and rainfall (mm) of the site during 2010 and 2011 are recorded.

Plant material: This investigation was aimed at studying the rooting ability and root quality of Arbequina olive cultivar. As plant material, a Spanish olive tree cultivar 'Arbequina' (*Olea europaea* L.) was selected for the establishment of high density olive plantation in Kingdom of Saudi Arabia (KSA). It's very productive and totally adapted to the super-intensive system and characterized by early entry into production and by high and constant productivity. Fat content is high and the quality of its oil is excellent, although not very stable. It makes soft and buttery oil ideal for blending.

In this study, selected bearing Arbequina trees were used as cutting sources and were subjected to adequate pruning and fertilization. Cuttings, made of semi hardwood shoots (one-year old wood) of lengths between 15 and 18 cm, were harvested on November 2010 at the morning and maintained moist until using. They were prepared by retaining 4 leaves and treated with hormone solution. The lower base of cuttings was dipped in IBA 4000 ppm concentration to a depth of 1 cm for 10 sec and with distilled water as a control treatment before planting.

Rooting conditions: The experiment was carried out in a controlled greenhouse where the air temperature was about 22+/-2°C, the relative humidity was kept at 50-70% and the temperature of the rooting substrate at 24-26°C as recommended by Hartmann *et al.* (1997). Cuttings were filled with sand, peat moss or perlite as rooting substrates and placed on 10 cm depth with 5 cm space between cuttings. Monitoring was done every three days to remove dead and infected leaves and cuttings. Three different rooting substrates: perlite and sand (inorganic substrates) and peat moss (organic substrate) were prepared with a number of about 200 cuttings in each substrate.

Data recording

Rooting ability: Samplings of semi-hardwood cuttings was performed 80-100 days after the beginning of rooting treatments and were conducted to the laboratory where they were washed with running water to remove the substrate covering the roots. A hand sprayer has been used to cause minimum damage to the roots. After that, each cutting was scored for rooting percentage, the percent of callused and necrosed cuttings, survival rate, mean number of roots developed by cutting, average root length of cuttings and number of days to root, as described by Hechmi *et al.* (2012). Cuttings with roots ≥1 mm were considered to have rooted and included for calculating mean number of roots. Root initials ≥0.5 cm were considered for calculating mean length of roots. Cuttings with roots =1 mm were considered to have rooted and included for calculating mean number of roots. Root initials ≥0.5 cm were considered for calculating mean length of roots.

Root morphology and development: This study was conducted to explore how adventitious roots initiate and develop in the cultivar Arbequina on the different substrates (perlite, sand and peat moss). Stem cuttings were treated with 4000 ppm IBA, or distilled water and sampled every 10 days over 60 first days to observe adventitious root formation and development and callus formation at the end of cuttings. The percentage of calli was the ratio between the numbers of calli developed to the total number of cuttings. The sampling ended about 80-100 days after planting as the cuttings had roots of an average length of 10 mm and the rooting process was considered over.

Statistical analysis: Ten cuttings were set per treatment and replicated three times and all obtained data were subjected to analysis of variance. Analysis of variance was performed to statistically examine the effects of substrates, IBA treatment and their interactions and to separate means.

RESULTS AND DISCUSSION

As the environmental conditions of mother plants and of cutting sampling are important factors for rooting success. The meteorological data was collected in the airport station of Al-Jouf region (KSA), near the mother plant field. Data showed little variation in climatic parameters in the years of sampling 2010 and 2011. The annual average temperature ranged between 25.2°C (2010) and 22.8°C (2011) and annual maximum and minimum temperature values remained stable from 2010 and 2011. It's evident that mother plants had to withstand more severe drought conditions with

24.9 mm distributed in 13 days and 18.79 mm distributed in 9 days yearly cumulative precipitation values. Climate Al-Jouf-Historical weather (http://www.tutiempo.net/en/Climate/Al-Jouf/403610.htm). All the mother orchard trees received perennial and supplemental irrigation.

The effect of treatments on rooting ability and quality of semi hardwood cuttings of Arbequina cultivar are summarized in Table 1. The percentage of callused and necrosed cuttings, the survival rate, the mean number of roots developed by cutting, the average root length of cuttings and the number of days to root were significantly affected by substrate properties, IBA concentration and their interactions.

Rooting ability and root growth: Root formation which is strongly stimulated by exogenous applied IBA at 4000 ppm, was dependent on the substrate used as rooting bed. The results showed that despite the relatively good root production obtained on sand, negative effects were seen in terms of root elongation and number of roots. It's evident from the data recorded for different substrates that cuttings planted in sand took least number of days to rooting (16.9), least mortality (15%), recorded high percentage of rooting (90%) and survival rate (98.7%) but gave low mean root number of 2.85 and low mean root length of 3.91 cm compared to perlite (Table 1). Isfendiyaroglu and Ozeker (2008) reached the same conclusions in Ayvalik olive cuttings; they concluded that pure sand reduced markedly root formation as well as the quality of the roots obtained.

Cuttings with better quality in terms of root length and thickness were achieved in perlite compared to peat moss and sand substrates (Table 1). Perlite gave the highest number of primary and secondary roots (8.06), the longest roots (5.76 cm) and the less number of days to rooting (19 days). It was found that those cuttings were healthy and grew well with high survival (88%), their root growth was also good. The roots are longer, more fibrous and more profusely branched and continued growing. This might be explained by the good water holding capacity and porosity of perlite. Similar findings were reported by Isfendiyaroglu *et al.* (2009), who suggested that better results in olive rooting were achieved when substrates with good aeration and reduced water retention capacity were used.

Using peat moss as rooting substrate significantly decreased root length and number per cutting; it exerted strong inhibition on primary and lateral root elongation. Also, peat moss induced roots 2 or 3 weeks later than roots induced by perlite. Root initiation in Arbequina semi hardwood cuttings, in this experiment occurred less than 3 weeks after IBA treatment on sand and perlite substrates, therefore about 4 weeks earlier than on peat moss (69.7 days from planting). The roots on peat moss were thicker and shorter, growing no further than 16 mm measured after 12 weeks whereas perlite roots were much better. Also on peat moss, exhibition of necrosis and formation of

Table 1: The effect of IBA and substrates on rooting ability and quality of Arbequina semi hardwood cuttings after 90-120 days

Substrate	IBA concentration	Survival cuttings (%)	Callused cuttings (%)	Necrosed cuttings (%)	Rooted cuttings (%)	No. of roots/cuttings	Average length of roots/cutting (cm)	Days to rooting
Sand	0	13.1±0.37	46.14±3.07	40.17±2.99	5.89±0.48	1.06±0.09	1.12±0.32	56.9±3.09
	4000 ppm	98.7±1.8	21.43±2.43	15.01±1.58	90.06±6.41	2.85±0.26	3.91±1.43	16.9±1.23
Peat-moss	0	14.2±0.98	44.81±3.52	54.71±7.53	0.86±0.12	0.13±0.04	0.25±0.08	69.7±3.55
	4000 ppm	44.3±0.71	58.1±5.820	46.42±4.01	27.51±1.08	1.11±0.17	1.63±0.16	37.1±2.10
Perlite	0	14.2±0.13	24.73±3.08	36.77±4.86	1.93±0.17	1.63±0.14	1.81±0.23	69.6±3.99
	4000 ppm	88.6±1.76	17.65±1.48	15.88±1.91	85.11±5.81	8.06±1.58	5.76±1.07	19.6±2.04

Values are Mean±SE all values are an average of 30 replicates, IBA: 1H-indole-3-butyric acid more callus at the base of cuttings which is undesirable for acclimatization were observed. Rooting ability was dramatically decreased when pure peat-moss was used (Table 1) which is considered by Loach (1988) too moist with limited aeration as substrate.

According to Hartmann *et al.* (1990), successful rooting is determined not only by rooting percentage but also by the number and the length of roots formed. These parameters were found to be highly dependent on IBA treatment and substrate type as rooting bed. The results indicated that semi hardwood cuttings without any hormone treatment (control cuttings) were able to root but the rooting percentage was low and the values for average number of roots, average length of roots were very less in comparison to IBA treated cuttings at 4000 ppm (Table 1). For the three substrates tested, the percentage of cuttings that rooted was significantly increased using 4000 ppm IBA which gave higher rooting, more developed roots and longer roots than the control (Table 1). Rooting hormones, increase overall rooting percentages, hasten root initiation, increase the number and quality of roots and encourage uniformity of rooting as suggested by Epstein and Wiesman (1987) and Daoud *et al.* (1989).

Root formation and quality were strongly suppressed in peat moss and these negative effects are likely related to its high water retained capacity which causes low aeration within the substrate affecting the oxygen diffusion to the roots (Abad *et al.*, 2002). Perlite constituted of inorganic materials with low water retention capacity and high aeration enhanced root initiation (Dvin *et al.*, 2011a). This probably explains why perlite recorded high rooting quality than the two other substrates sand and peat moss. It's in accordance with (Ozenc and Ozenc, 2007) who noted that inorganic materials were found to be more suitable for rooting and organic materials were more effective in root growth.

The logical conclusion seems that large numbers and high length of roots are associated with adequate nutrient absorption from the substrate. The results obtained are in contrast as the highest number and length of Arbequina cuttings are recorded in perlite substrate which is inorganic and characterized by poor absorption of nutrients. The situation may be comparable to the findings of (Ozenc and Ozenc, 2007) on kiwifruit cuttings who observed that thick roots were higher in inorganic substrate, on the contrary, fine roots were higher in organic substrate.

Callus formation and root development: Olive vegetative growth characteristics were determined by studying rooting, percent survival and callus development and those parameters are very important for the quality and survival of the new plants (Gerrakakis and Ozkaya, 2005). The results revealed that IBA treatment combined to sand or perlite had a significant effect on the final percent of the rooted cuttings, on the survival of cuttings and mainly they increased the percent of rooted cuttings without callus (Table 1). The most callus formation was observed on peat moss substrate with a rate of 58.1%. This root system with callus proved less qualitative during propagation, because the cuttings with such root system developed significantly less roots per rooted cutting (1.11) and their root length was shorter (1.63 cm) than those of the cuttings without callus. In addition and when cuttings were treated with IBA 4000 ppm, it was observed that callus formation was 21.4% in sand and 17.6% in perlite substrate while in control (cuttings dipped in water), it was 46.1 and 24.7%, respectively.

In untreated control cuttings, high callus formation was also abundant in cuttings which necrosed and died at the end of the growing season. A late emergence of roots (69.7 days) and significant development of fewer roots per rooted cutting (0.13) were observed; their average root growth was weaker (0.25 cm) than those of the cuttings without callus. Figure 1a shows the

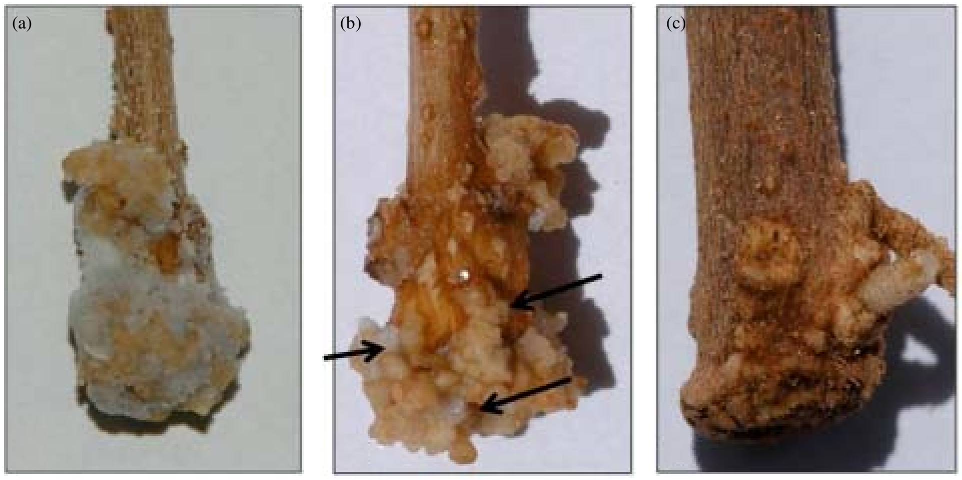

Fig. 1(a-c): Callus formation and root emergence at the base of Arbequina cutting during the first 60 days of planting (a) Marked callus formation at the cut surface leading to the emergence of roots and weaker root growth in control, (b) Small protuberances and adventitious roots formed around the wounded end of cuttings 4-5 weeks after planting (arrows) and (c) After that, progressive root elongation was observed

marked callus formation at the cut surfaces and the absence of root formation in control. Data reported in Table 1 shows a significant difference in callus formation and rooting of Arbequina cuttings because the highest rooting percentages were not associated with the greatest increase in callus formation during the propagation period. It is in agreement with Hartman and Kester (1975) who noted that the formation of callus and formation of roots are independent of each other.

It's clear that the establishment of an adventitious root system in Arbequina cuttings is related to different factors. The suitable IBA concentration of 4000 ppm and perlite prevented callus appearance and rapidly promoted formation and development of adventitious roots in the cuttings of Arbequina cultivar. Results are also supported by Ozenc and Ozenc (2007) who indicated that not only a suitable IBA concentration of 4000 ppm but also inorganic media components which may prevent callus formation in olive.

This study revealed that Arbequina roots can arise from the callus tissue (adjacent to the stem) or directly from the stem tissue. Callus development at the basal end of Arbequina cuttings mainly occurs during the first 60 days and after 6-8 weeks from planting, root initiation can be observed. This newly formed callus consisted of irregular unspecialized parenchymatous cells and in olive; this callus proliferated from vascular cambium and in part from phloem and xylem parenchyma (Ayoub and Qrunfleh, 2006). Cuttings with excellent callus often failed to root while many cuttings with poor callus developed root primordial and elongated. Ayoub and Qrunfleh (2006) explained that callus formation in the cortex of the cutting and thickness of the cortex may act as a barrier for the future development of the root primordia and may result in failure of root primordial to emerge in olive cuttings.

Root morphology and development: The root system of Arbequina olive cuttings developed was compared in the three substrates: sand, perlite and peat moss. When the cuttings were removed from the substrate after 2-3 weeks planting according to the substrate, no visible modification of their morphology could be detected at the base of cuttings (Fig. 1b). Afterwards and 3-4 weeks after planting, small enlargements had the appearance of small protuberances and 4-5 adventitious roots arranged radially around the base of cuttings (Fig. 1c). After that, it was possible to detect a progressive elongation of these roots which were more than 10 mm long on average. The root system was already formed 4-6 weeks depending on the substrate and IBA treatment. In the last period of rooting, the rooted cuttings were then ready to be transferred outside.

Differences in growth of roots is clearly visible in Fig. 2 which reveals the growth of roots in the 3 different substrates used in this experiment, 12 weeks after planting of rooted cuttings. Sand substrate developed a root system of cuttings where a main root was developed from which fine and few lateral roots (Fig. 2b). It produces root systems with long and poorly branched surface roots, whereas perlite substrate produce more vigorous well-branched roots that may go deeper into the soil when planted in the field. Also, perlite developed very shallow, widespread and heart rooting systems (Fig. 2a) which are very important to maximize rainfall interception near the soil surface in the field. Perlite substrate promotes more extensive and potentially deeper root system, so roots may well exploit water in the arid conditions of Al Jouf (KSA).On the contrary, peat-moss substrate gave a root system where smaller roots were developed horizontally (Fig. 2c). The finer root system of Arbequina cuttings on peat moss could be the reason for the shorter side root length as compared to perlite. It is in agreement with Day *et al.* (2010) who noted that physical properties and aeration influence the need for roots to go to greater depths and may restrict rehability to grow deeper. It is known that rooting system of cuttings developed in substrate; influence the tree rooting habit in the field (Crow, 2005). On the other hand, IBA combined to perlite influenced the development of acrobasal type of the rooting system and induced higher number of roots while IBA and sand influenced the development of basal type of rooting system (Fig. 2a, b).

Fig. 2(a-c): Rooting system of Arbequina cuttings developed in perlie, sand and peat moss substrates after dipping in 4000 ppm IBA solution and 12 weeks under mist humidification in Al-Jouf (KSA) (a) Rooted cuttings with vigorous well branched roots of good quality developed on perlite and development of acrobasal type of the root system (arrows); adventitious roots formed at node above the base, (b) Development of basal root system in the end of cuttings planted in sand substrate with long and poorly branched roots, root system developed a single axis and fine and few roots and (c) Fewer number of roots with low quality, produced on peat moss substrate showing necrosis at the end of cuttings

Arbequina cultivar recorded the lowest rooting cuttings rate (27.5%), highest mortality (54.7%) and the maximum days to rooting (37.5%) on peat moss and in presence of IBA (4000 ppm). The inability of Arbequina cuttings to root on peat moss may be attributed to 2 reasons: first, the changes in anatomical structure of the cutting during the rooting period due to variations in climatic conditions. The difficulty in olive rooting was partially attributed to the increase in cortex thickness during rooting forming mechanical barrier to emergence of root initials (Ciampi and Gellini, 1963; Ayoub and Qrunfleh, 2006). The other possible reason may be that the high rooting percentage obtained in this experiment (more than 80%) could be attributed to favorable physiological conditions when cuttings were taken from the tree. In fact, the callus was formed during winter (December-15 January in KSA) and the rise in temperature from 15 January to 15 February stimulated root development. This is in agreement with findings of Ahmed *et al*. (2002) who suggested that ability or inability of olive cuttings to root may be attributed to the physiological climatic conditions which prevented or not growth hormones to stimulate the rooting.

Time of rooting, survival rate and necrosis of cuttings: Arbequina cuttings kept their survival rate in all the substrates tested. At 4000 ppm, cuttings showed a high survival rate of 98.7% in sand and 88.6% in perlite substrates and low rate of 44.3% in peat moss while in control. Arbequina cuttings kept their survival rate in all the substrates tested. At 4000 ppm, cuttings showed a high survival rate of 98.7% in sand and 88.6% in perlite substrates and low rate of 44.3% in peat moss while in control, lowest alive cutting (less than 15%) was recorded. It is important to note that on peat moss, some of cuttings developed neither roots nor callus but still remained alive. Gerrakakis and Ozkaya (2005) reached the same conclusion in olive and noted that the important role of substrates in affecting not only the formation of roots and callus but also the survival rates of olive cuttings.

As indicated in Table 1, mortality of cuttings, due to necrosis, differs among the three substrates tested and IBA concentration, it ranged from 46.42% in peat moss to 54.71% in control. A defoliation phenomenon of cuttings just before necrosing and dying on peat moss substrate were noted. Low values of mortality enregistered on sand (15.01%) and on perlite (15.88%) may correspond to the nature of perlite and sand which were inorganic materials. The maximum time to root for Arbequina cultivar was enregistered for cuttings dipped in 4000 ppm IBA and planted on peat moss substrate while the minimum time (less than 20 days) to root was recorded in sand and perlite substrates. It has been observed that number of roots and the time required for the emergence of first roots was inversely correlated. More the number of roots, less the days required for rooting. This situation may be attributed to some internal factors such as accumulation of some inhibitors level in the cuttings as suggested by Hartmann *et al*. (1997) on olive cuttings and Awan *et al*. (2003) on peach rootstocks.

CONCLUSION

Some studies are available about the ability of rooting potential in Arbequina olive cultivar, therefore the current study was conducted to study the rooting morphology and growth of the cuttings using the combination of IBA treatment at 4000 ppm and 2 types of substrates: inorganic (perlite and sand) and organic (peat moss). The use of mist system and semi hardwood cuttings of Arbequina olive cultivar under the Al Jouf conditions associated to perlite substrate and 4000 ppm IBA concentration is a successful method to have successful root formation and root spread. Results obtained can be used to improve practices, to increase the understanding of the dynamics of root development in olive. But this study needs some more studies in the future to determine root system development of the new plants under different soil conditions and tree spacing mainly under high density system in arid conditions of KSA.

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