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Performance of Olive Cuttings (*Olea europaea* L.) of Different Cultivars Growing in the Agro-climatic Conditions of Al-Jouf (Saudi Arabia)

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ABSTRACT
In order to enhance the establishment of olive plantation in the Kingdom of Saudi Arabia (KSA), an investigation was carried out to improve the rooting ability of olive cuttings. This research aimed to study the effect of three substrates (sand, peat moss, perlite) and IBA treatment (at 0 and 4000 ppm) on rooting ability of three olive cultivars (Arbequina, Koroneiki and Picual). The percentage of cuttings that rooted, the number of roots produced by cutting, the average root length and the survival rate were recorded. These parameters were significantly influenced by the interactive effect of substrates, cultivars and IBA treatment. The semi hardwood cuttings planted in perlite and sand had higher rooting percentage than those planted in peat moss with a significant difference between cultivars. Very low rooting occurred in cuttings dipped in distilled water (control) for all cultivars and in all substrates. At 4000 ppm IBA, Arbequina and Koroneiki cultivars gave high results while Picual cv. recorded worst performance in all the substrates tested. Results in relation to the physical properties of the substrates used in this experiment were also discussed.

Key words: Rooting substrates, semi-hardwood cuttings, olive cultivars, root number, root length

INTRODUCTION
Olive is a recent introduction in Kingdom of Saudi Arabia, becoming increasingly popular among growers mainly in Al-Jouf region (Northern part of the Kingdom). Olive has an enormous potential in KSA markets and the demand for rooted cuttings for planting new orchards is increased. Also, olive orchards in this region, due to the adaptability to the region's climate, are more and more developed (Mehri, 2009).

Methods to propagate olive cultivars are numerous but semi-hardwood cuttings are one of the least expensive and easiest methods of vegetative propagation (Hartmann *et al*., 2002; Dvin *et al*., 2011b). Cuttings are easy to prepare and require little or no special equipment during rooting (Fontanazza, 1996). Also, propagation from cuttings produces a plant with the same characteristics as the parents and maintains desirable fruiting traits. Previous researchers reported that the biggest problem in olive is the low ability of regeneration leading to low percentage of rooting (Fabbri *et al*., 2004). Rooting ability of olive cuttings is influenced by a number of factors related to cultivars (Fouad *et al*., 1990; Turkoglu and Durmus, 2005), age of source tree and prelevement date of cuttings (Sebastiani and Tognetti, 2004; Ahmed *et al.*, 2002; Hartmann and Loreti, 1965), type of cuttings (Loreti and Hartmann, 1964; Turkoglu and Durmus, 2005), rooting media (Isfendiyaroglu *et al.*, 2009; Awan *et al.*, 2003) and the treatment concentration of auxin-like compounds (Hartmann and Kester, 1975; Loreti and Hartmann, 1964; Hartmann *et al.*, 2002; Asl Moshtaghi and Shahsavar, 2011).

The physical properties of the rooting substrate influences greatly the olive percentage rooting and the quality of roots and perlite is the most used rooting substrate in olive producing countries. Seasonal variation in rooting ability was also reported for different cultivars and may be attributed to the physiological status of cuttings tissue (Nahlawi *et al.*, 1975) and to stem anatomical differences between cultivars (Fouad *et al.*, 1990; Ayoub and Qrunfleh, 2006). Experiments on the rooting of olive cuttings conducted by Rio *et al.* (1991) showed that the availabihty and mobilization of carbohydrates towards the basal of cuttings were essential for rooting.

The aim of this work was to study the rooting capacity of three olive cultivars planted in Al-Jouf, northern part of KSA. Three rooting substrates (sand, perlite and peat:moss) and semi hardwood cuttings of 3 olive cultivars (Arbequina, Koroneiki and Picual) dipped in 4000 ppm IBA in a factorial design were used.

MATERIALS AND METHODS

The present studies were carried out at olive research unit located in the research center of Al-Jouf, KSA during 2010-2011. The center is located at an altitude of 684 m above sea level, latitude 29.8°N and longitude 40,1°E. The area receives an average annual rain fall of 24.9 mm in 2010 and 18.79 mm in 2011 with average maximum and minimum temperature of 29.7 and 15.8°C in 2011, respectively. Climate Al-Jouf-Historical weather (http://www.tutiempo.net/en/Climate/Al-Jouf/403610.htm).

The cultivars included in this study (Arbequina and Picual from Spain, Koroneiki from Greece) were selected for their use in the high density orchards and were studied to assess the influence of cultivar and substrate on root ability. Arbequina is a smaller Spanish cultivar used primarily in high-density plantings. It is very productive and totally adapted to the super-intensive system and comes into production at an early age with good and constant yields. Fat content is high and the quality of its oil is excellent, although not very stable. Picual is the most widely planted Spanish cultivar with a cold hardy tree that makes strong and spicy oil. Koroneiki is a Greek cultivar where it is also known as Koroni, Kritikia, Ladolia and Psylolia. It is popular high-density tree known for its high removal force with a very early entry into production. A cultivar of interest for super intensive plantations, it produces quality oil that is both flavorful and pungent. Fat content is around 20% with excellent oil quality and very stable.

Cutting treatments: Study was carried out under mist system in greenhouse conditions. Olive cuttings were collected on the end of November 2010 from selected stock plants growing at the olive research unit of Al-Jouf, KSA. The leafy semi hardwood cuttings were prepared from vigorous one-year shoots of olive about 6-7 mm diameter and 15-18 cm length. The sub-terminal cuttings with 2 pairs of leaves were used. The cuttings were taken from 3-year-old mother plant. The basal end of cuttings was dipped in 4000 ppm IBA for 10 sec before planting in 40×20×11 cm root flats filled with 3 different media: sand, perlite and peat-moss. All the cuttings were placed in basal heated benches that were filled with substrate maintained at a constant temperature of 23±2°C.

The bottom of the rooting bench was heated at 24°C, the temperature was 22+/-2°C and the relative humidity was kept at 50-70%. The cuttings were irrigated with mist system every hour for 10 sec to maintain microclimate conditions for rooting and survival.

To test the effect of IBA application, the cuttings were dipped in IBA solution at 4000 ppm. Indol-3-butyric acid (IBA) solution at 4000 ppm was freshly prepared dissolving IBA powder (Sigma, St Louis, MO, USA) in an alcohol/water/glycerol (2.5:6.5:1 v/v/v) solution. For control, basal ends were treated with distilled water.

Rooting score: Sampling of semi-hardwood cuttings was performed 80-100 days after the beginning of rooting treatments and each cutting was scored for the rooting percentage, number of roots/cutting (number of primary and secondary roots produced), the mean root length/cutting (cm) and cutting survival. The percentage of rooting was calculated as the ratio of the number of rooted cuttings to the number of total cuttings x 100. The mean of the roots length in each treatment was measured as the total number of roots length in each treatment divided by the number of cuttings of that treatment. Only the roots length more than 0.5 cm was measured. The average number of roots per cutting was calculated as the total number of roots divided by the number of the cuttings of that treatment. All the parameters were scored in every experiment for the three cultivars: Picual, Arbequina and Koroneiki and on all the substrates: sand, peat moss and perlite.

Statistical analysis: This experiment consists of three replications and each replication consisted of 10 cuttings. At sampling date, analysis of variance was performed to study the effect of the substrate, cultivar and their interactions and to separate means. Significant differences were determined with Duncan's multiple range test at $p<0.05$.

RESULTS

Olive cuttings taken from different cultivars (Picual, Arbequina and Koroneiki) were studied at the olive research center of Al-Jouf, KSA to assess the influence of substrate and cultivar on root ability of olive cuttings during the year 2010-2011. The leafy semi hardwood cuttings were taken from 3 years old of three olive cultivars, dipped in 4000 ppm concentration for 10 sec before planting on beds prepared from sand, perlite and peat-moss separately.

Changes in rooting ratio of different rooting substrates and cultivars are shown in Table 1. The analysis of variance indicated that the percentage of cuttings that rooted, the mean number of roots produced per cutting, average root length and survival of rooted cuttings were significantly affected by rooting substrates, cultivar and IBA treatment and their interactions. On control trial, the minimum rooting rate, root length and root number were recorded in cuttings dipped in distilled water. With IBA concentration at 4000 ppm, the percentage of rooted cuttings varied between 1.5% and 90% depending on the cultivar and on the kind of rooting substrates tested (Table 1).

Effect of substrate on root production and quality: An analysis of variance of the results showed significant differences in rooting and the optimum root elongation and root number were strongly dependent on substrate used. The best substrates in term of rooting percentage consisted of sand and perlite. Peat moss comprised the worst substrate which appeared to be highly limiting to the rooting and development of cuttings. When cuttings were treated with 4000 ppm IBA and planted in sand or perlite, best results in terms of rooting percentage were enregistered, this is dependent on the cultivar.

Table 1: Effect of substrates on rooting ability of olive cultivar cuttings dipped in 0 and 4000 ppm IBA

Hormone dose		Rooting rate (%)		Average number of roots/cutting		Average root length/cutting (cm)		Survival rate of rooted cuttings (%)	
Substrate	Cultivars	0 ppm	4000 ppm	0 ppm	4000 ppm	0 ppm	4000 ppm	0 ppm	4000 ppm
Sand	Picual	3.8±0.660	17±2.54	0.63±0.03	1.8±0.880	0.04±0.011	1.83±000	9.7±0.08	44.9±0.74
	Arbequina	5.89±0.48	90.06±6.41	1.06±0.09	2.85±0.26	1.12±0.323	91±1.430	13.1±0.37	98.7±1.80
	Koroneiki	7.01±1.99	76±4.03	1.12±0.83	2.7±0.900	0.97±0.243	1±1.1100	18.8±3.07	91.3±2.01
Peat moss	Picual	0.3±0.070	1.5±0.67	-	0.7±0.280	-	0.73±0.33	4.3±0.32	13.4±1.06
	Arbequina	0.86±0.12	27.5±1.08	0.13±0.04	1.1±0.170	0.25±0.08	1.63±0.16	14.2±0.98	44.3±0.71
	Koroneiki	1.17±0.26	14.5±1.81	0.47±0.05	0.9±0.600	0.31±0.09	1.33±0.76	9.08±0.11	50.1±1.60
Perlite	Picual	0.7±0.200	10±0.45	0.31±0.07	1.9±0.920	0.19±0.12	1.59±0.82	8.6±0.09	31.7±0.16
	Arbequina	1.93±0.17	85.1±5.81	1.63±0.14	8.06±1.58	1.81±0.23	5.76±1.07	14.2±0.13	88.6±1.76
	Koroneiki	2.8±0.850	78±3.98	2.19±1.02	6.31±1.55	1.16±0.92	5.04±2.06	19.7±0.74	98.01±1.04

According to experimental results, it is evident from the data recorded for the 3 rooting substrates, that sand substrate gave best results with regard to percentage rooting, while perlite developed highest root number per cutting and root length. Perlite was more efficient on elongation and subsequent root emission than the sand rooting substrate; it exhibited a large number of root hairs. Cuttings in perlite had longer roots than they had on sand and peat moss and than control did in all substrates.

Root number and root length were significantly higher in perlite substrate for all the cultivars followed by sand indicating a positive correlation between number of roots and root length in these cultivars tested. Root length was the highest in all cultivars on perlite substrate followed by sand as the best effective treatments. The least effective substrate on root length and root number was peat-moss where we recorded the shortest and the thinnest roots and least average number of roots per cutting. Peat moss resulted also in low rooting rates comparing to perlite and sand, the rooting rates were only 1.5-27.5% depending on the cultivar. In data reported in Table 1 and compared to the control, maximum number (8.06) and maximum length (5.76 cm) of roots were obtained with IBA at 4000 ppm and perlite substrate while peat moss produced the lowest number (0.7) and the lowest length of roots (0.73 cm). Peat moss was found to be unsuitable because it produced large swellings at the base of cuttings and occasional apical necrosis. Planted in peat moss substrate, cuttings exhibited degenerative changes and necrosis even with IBA application at 4000 ppm (Table 1).

Morphological characters of the roots produced are also different according to the substrate used. Data concerning cutting survival shows that maximum percentage survivals were obtained when cuttings were dipped in 4000 ppm IBA and planted on perlite and on sand substrates and minimum plant survival were obtained in control treatment (cuttings dipped in distilled water) and on peat-moss rooting bed. Sand recorded maximum survival percentage more than 90% for Arbequina and Koroneiki cultivars and 44.9% for Picual cv. while peat moss substrate failed to support survival of cuttings mainly when dipped in distilled water. Peat moss recorded 33.4, 44.3 and 50.1% survival for Picual, Arbequina and Koroneiki. cultivars (Table 1).

In this experiment, cuttings not subjected to IBA treatment seem to maintain their survival on perlite substrate, 8.6, 14.2 and 19.7%, respectively for Picual, Arbequina and Koroneiki cultivars. Table 1 indicated that low survival was recorded for Picual cultivar. We noted for the latest, survival rates of 31,7%; 33%; 44,9% in perlite, sand and peat moss, respectively. Maximum survival percentage was achieved by Koroneiki cv. planted on perlite (98%) and on sand (91.3%).

Effect of cultivars on rooting production and quality: Of the three different substrates tested in the present work, sand and perlite substrates showed very high rooting ability, compared to peat-moss but these results depend on the genotype tested. Cultivars revealed significant differences for the percentage of rooting, root length and number. Cuttings taken from Arbequina and Koroneiki cultivars. gave higher rooting percentage, more number of roots per cutting and longer roots than Picual cv. For example, on perlite substrate, the percentage of cuttings that rooted was about 85 and 78%, number of roots was about 8.06 and 6.31 and root length was about 5.76 and 5.05 for Arbequina and Koroneiki cultivars., respectively. While, on the same substrate, less rooting percentage (10%), least root number (1.9) and shortest roots (1.59 cm) were detected in Picual cultivar cuttings.

In all the substrates tested, Arbequina cultivar proved more effective in terms on the rooting percentage, the number and the length of roots. The percent rooting was 90; 85 and 27.5% on sand, perlite and peat moss, respectively. The maximum rooted cuttings with the highest survival rates were observed also in Arbequina cv. on sand (90 and 98.7%, respectively) which were significantly better over all the substrates tested. While, the lowest values were recorded with Picual cv. on peat moss (27.5 and 44.3%, respectively). Koroneiki cuttings cv. planted on perlite, caused 78% rooting with corresponding survival rate of 98.01% (Table 1).

Successful rooting is determined not only by rooting percentage but also by the number and the length of roots formed (Hartmann *et al.*, 1990). Comparison of rooting capacity between sand and perlite substrates, revealed that Arbequina cv. rooted better on sand (90%) but more roots grew in perlite with greatest root number and the highest root length. The Koroneiki cultivar was the second best to give good response to 4000 ppm level. For the later, high rooting (78%), high number of roots (6.31) and lengthy roots (8.04 cm) were achieved on perlite. Picual cv. showed maximum number of 1.9 roots per cutting and maximum length of 1.83 and 1.59 cm on sand and perlite substrates.

Effect of IBA on root production and quality: Preliminary trials on a wide range of IBA concentrations had shown that the dose of 4000 ppm gave the best results as regards both rooting rates and the number of roots per cutting, regardless of cultivars planted in Al-Jouf conditions, KSA (data not published). Data in Table 1 revealed that increasing IBA concentration from 0 to 4000 ppm significantly improved rooting rate, root number and root length per cutting in the three olive cultivars in comparison with control.

For all the cultivars, the control treatment gave the lowest values because in the absence of IBA, cutting rooting in sand substrate reached 5.89% in Arbequina cv. 3.8% in Picual and 7.01% in Koroneiki cv. When, IBA was applied, the same substrate gave significantly better results in terms of rooting percentage and quality of roots (90, 17 and 76%, respectively for Arbequina, Picual and Koroneiki cultivars). Maximum number of roots per cutting was recorded when cuttings of Koroneiki cv. were treated with 4000 ppm IBA concentration and planted in sand (2.7/cutting) and in perlite (6.31/cutting) while minimum number of roots were noted in control (1.12 and 2.19 per cutting, respectively). At the same concentration of IBA, Picual cv. exhibited the least root length compared to Arbequina and Koroneiki cultivars.; 1.83 cm in sand, 0.73 cm in peat moss and 1.59 cm in perlite. When Picual cuttings were dipped in distilled water, the values were about zero.

DISCUSSION

In the conditions of Al-Jouf and under mist conditions, our findings revealed that perlite gave the best values in terms of length and number of roots by cuttings and sand recorded best rooting. These results of the most evident stimulating effects of substrate on the rooting of cuttings in olive suggested using the two-component mixtures. The use of mixture of perlite and sand as substrate can improve rooting, root length and root number, this, may be due, as suggested by Awan *et al.* (2003) to the improvement of both aeration and water retention capacity. Moreover, the use of two substrates is costly and time consuming.

Increased rooting rates due to the use of different substrates have been reported for olive cuttings (Awan *et al.*, 2003; Isfendiyaroglu *et al.*, 2009), for apple cuttings (Dvin *et al.*, 2011a, b) and for kiwifruit cuttings (Ozenc and Ozenc, 2007). To determine the most successful media among those traditionally used, Isfendiyaroglu *et al.* (2009) used 25 different substrates pure or mixtures and concluded that sand-perlite (1:2 v/v) led to more than 90% of rooting while pure sand, peat and peat-sand mixtures gave very low (5-28%) rooting percentages of Ayvalik olive cuttings. Also pure perlite and its mixture with sand in 2:1 ratio appeared to be the best component for rooting olive cuttings. The optimum rooting quality for all cultivars was enregistered on perlite substrate, as previously reported by several researchers on olive propagation (Awan *et al.*, 2003; Isfendiyaroglu *et al.*, 2009). Perlite has been shown to increase rooting of several species because it is recognized to have a unique capillary action and to increase aeration and drainage within the container because of its uniformity and lightness (Paradiso and de Pascale, 2008).

Many researchers noted that good water management of substrate is crucial for rooting success of cuttings; the main physical properties of different substrates are related to water content, aeration capacity and pH. To promote root formation and growth, De Boodt and Verdonck (1972) defined the aeration capacity of an ideal substrate, this should be about 20-25%; it was suitable for perlite (20,4%) and inadequate for peat-moss (16,2%). Water content of media is also an important parameter to enhance rooting; the substrate must be kept moist enough to promote root growth. Ozenc and Ozenc (2007) signaled that an over water can cause anaerobic conditions leading to less rooting rate as it may be signaled on peat-moss.

Significant cultivar differences were observed on peat moss substrate where maximum rooting rate varied from 1.5% in Picual cv.; 27.5% in Arbequina cv. and 14.5% in Koroneiki cv. Peat moss has been recognized to have high water holding capacity which causes poor air-water relationship, leading to low aeration within the substrate (Isfendiyaroglu *et al.*, 2009).

Experiments showed that the lowest rooting percentage was obtained in peat moss substrate. This might be because peat moss contains more organic and mineral nutrients compared to perlite and sand which are inorganic substrates. Peat-moss substrate has 65,8% of organic matter content, 1.1% total organic nitrogen, total organic matter of 75% (Piedra *et al.*, 2005), 125 mg kg^{-1} Phosphore and 155.5 mg kg^{-1} Potassium. On the contrary, perlite contains 0% of all these elements; it's known that the most of granular inorganic substrate such as perlite have low water retention capacity (Dvin *et al.*, 2011b) and were used for its porosity (Ozenc and Ozenc, 2007). Studying the influence of rooting substrate on apple cuttings, Dvin *et al.* (2011b) indicated that inorganic material were found to be more suitable for rooting, however, organic materials were more effective in root growth (Ozenc and Ozenc, 2007). Also, pH value was 7.3 for peat-moss which was high and inadequate for rooting, because according to Latigui *et al.* (2011), it exceeded the recommended range for healthy rooting, which was between 5.5 and 6.5.

According to Argo (1998) and Richards *et al.* (1986), a good growing medium would provide sufficient support for the plant serve as a reservoir for nutrients and water, allow oxygen diffusion to the roots and permit gaseous exchange between roots and atmosphere. Lower percentages of rooting of the cuttings of the three cultivars on peat moss can be resulted in a lack of one or more of the beneficial characteristics of the medium used. Also, minimum survival percentage has been recorded in peat moss due probably to the nutrients contained or to high water holding capacity which provided favorable condition for fungal growth (Awan *et al.*, 2003). Dvin *et al.* (2011a) signaled that peat moss has a high water holding capacity which causes poor air-water relationship, leading to low aeration within the substrate and suggested that incorporation of perlite material into peat moss could improve the aeration status of the media.

Our results showed that pure sand gave good results in terms of rooting percentage. However, our findings contrast those of Gerrakakis and Ozkaya (2005) who reported the rooting and the survival rates of “Ayvalik” olive cuttings to be reduced in sand. For Isfendiyaroglu *et al.* (2009), the poor rooting of olive cuttings in this substrate may be linked to the rapid loss of water from this substrate. For Awan *et al.* (2003), the type of rooting medium had no effect on rooting in terms of root length but weight per cutting was greatest in sandy loam.

High significant cultivar effect was also signaled in this work, a genetic variation was reflected in the differences observed among the cultivars in their response to substrates as signaled by Awan *et al.* (2001). The cultivar differences in the rooting ability of olive cuttings have been linked by Ozkaya and Celik (1999) to the endogenous carbohydrate status. The inability of Picual cuttings to root may be attributed to the physiological conditions of plant material planted under the conditions of Al-Jouf. According to Ahmed *et al.* (2002), at moderate temperature, the cambium region is divided actively and physiological condition of the cambium for root initiation and development was suitable.

Overall, 4000 ppm IBA gave the best rooting frequencies for the three cultivars and for all substrates used. In our experiments, it is found that hormone application is a stimulating factor and the concentration of 4000 ppm used is found to be the best for rooting for the 3 olive cultivars tested; Picual, Arbequina and Koroneiki. Many publications have summarized the usefulness of auxins as rooting hormone for cutting propagation and indicated that IBA is considered to be a well rooting auxin in olive cuttings (Hartmann *et al.*, 2002; Isfendiyaroglu and Ozeker, 2008). Although the dosages of IBA at 4000 ppm are suggested for two Iranian olive cultivars (Asl Moshtaghi and Shahsavar, 2011) and for Domat and Ayvalik cultivars. (Gerrakakis and Ozkaya, 2005). The IBA effect on rooting have been studied also for its effect on cell wall turgidity which accelerates cell division (Rahman *et al.*, 2002), increases cambial activity and differentiation of root primordial (Davies and Joiner, 1980). It is in agreement with Awan *et al.* (2003), who signaled the lowest rooting of olive cuttings recorded in control treatment (without IBA) due to minimum exposure of the cambium to develop root primordial. Mousa (2003) suggested that the effectiveness of auxin to raise rooting percentage of olive cuttings could be through increasing cambial activity and differentiation of root primordial or by mobilization of some auxin cofactors towards the base of the cuttings.

The previous study showed that apart IBA and rooting substrate, consideration variability was related to the cultivars. Therefore, further studies should be carried to plant the most promising varieties in field to study their tolerance to Al-Jouf environments (prevailing arid conditions) and to observe the root development and the root system distribution of plant in the soil according to planting density. Additional long term research is needed to establish a relationship between relate results developed from mist system studies with field conditions because possible benefits are considerable. In addition, investigation of the olive yield may allow for selection of trees that can better exploit limited soil resources in arid conditions and under different planting density.

CONCLUSION

Results of present study showed that under the agro-climatic conditions of Al-Jouf (KSA), rooting success is greatly affected by the substrate, the cultivar and exogenous IBA application. Arbequina and Koroneiki cultivars produced roots easily and quickly while Picual cv. had difficulty doing this. In conclusion, a selection program is recommended for rooting ability to select easy to root cultivars from our mother stock planted in fields and containing a selection of olive tree varieties from Italy, Tunisia, Syria, Spain, France, Greece and Italy. This selection can use their advantages in high-density orchard systems in the climatic conditions of Al-Jouf area (in the northern part of KSA) where there is an increase interest to olive plants.

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