Research Article

Effect of Timing Intervals on the Rooting Response and Performance of Olive Cultivars through Air-Layerage

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Abstract | The effect of timing intervals on the rooting response and performance of olive cultivars through Air-Layerage was determined at Olive Model Farm, Sangbhatti Mardan-Pakistan during the year 2014. The experiment was laid out according to Randomized Complete Block Design with two factors factorial arrangement. Air layerings were practiced on July 1, 16, 31; August 15, 30 and September 14 in plants of olive cultivars Frontoio, Manzanilla, Ottobratica, Pendolino and Picual. Significant variations were recorded among olive cultivars regarding asexual propagation through air-layerage. The daughter saplings of cultivar Manzanilla took less number of days (47.94) to root appearance, produced more rooting percentage (38.89%), number of roots (4.31), root length (4.61 cm) and root weight (1.77g) with more number of re-sprouts (3.18) and shoot length (5.28 cm). The time intervals of air layering also significantly influenced the rooting and growth attributes of olive cultivars. Mid August, proved to be the most favorable time for air-layering in terms of rooting percentage (42.40%), number of root (4.37), root length (4.35 cm), weight (1.68 g), number of re-sprout (3.67) and shoot length (5.61 cm) while late (September 14) and early (July 1) layering responded poorly to all the studied attributes. The response of individual cultivar was dissimilar to different times of layering. Cultivars: Frontoio, Manzanilla and Pendolino took minimum days to root appearance, attained high rooting percentage, produced maximum number of roots, root length and root weight with more number of lengthy re-sprouts, when air-layered during mid August while Ottobratica and Picual, layered during mid and late July respectively, responded well to all these attributed.

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Introduction

Olive belongs to family *Oleaceae* and genus *Olea* which includes at least 30 species, have the same chromosome number (2n = 46) and crosses between many of them have been successful. It is one of the important and oldest fruit tree in the world (Standish, 1960) and is successfully grown in areas having mean annual temperature 15-20°C, with a minimum of 4°C and maximum of 40°C (Therios, 2009). More than 11 million hectares of olives are grown in the world and about 98 percent of the world's olives are produced in the Mediterranean region. At present, olive oil is consumed in over 160 countries and the production during 2012 was 3.1 million tonnes which represents 1.7 percent share of total output of edible vegetable and animal fats (184 million tonnes). These figures highlight the strategic economic importance of the olive oil sector and its influential position in the international arena in terms of production and consumption

(Jean-Louis et al., 2015). The quantity of olive oil and table olive production was 3.25 and 2.66 million tonnes respectively, during 2013-14 (IOC, 2016). The areas having long warm summer and mild winter are suitable for the production of best quality olives and commercial production is found in belts around the world between 30°- 45° north and south of equator. Pakistan is located in the same region have varied climatic and soil factors, due to which all sort of temperate, subtropical and tropical zone fruit trees can be grown. Olive being a sub-tropical can be grown successfully in the subtropical mountainous region of Khyber Pakhtunkhwa and Balochistan (Baloch, 1994). According to the International Olive Council (IOC) estimates, the olive growing areas in the globe are rapidly increasing and reached 10,000,000 acres in 2012, against 9,100,000 in 2000. But unfortunately economically important olive cultivars, having intermediate or even poor rooting capacities, are found in most olive producing countries (Fabbri et al., 2004) and rooting in difficult-to-root cultivars is also influenced by colonelvariation (Loreti and Hartmann, 1964; Fernandes et al., 2002).

Olive is propagated through sexual and asexual means but the sexual method is usually not recommended, because seedlings are not true to type and the juvenile period is too long to bear fruit. Asexual or vegetative propagation through cutting, budding and grafting are the most widely used commercial techniques of propagation in olive. Air-layering has also been proven as an effective tool in a wide range of difficult to root species and can add to the value. The technique is usually practiced when the climate is humid and the stock is at active growth period (Wilson, 1974), the survival, branching growth and establishment can be promoted through various factors (Sileshi et al., 2007). Keeping in view the importance of air-layering and its timing in olive propagation the present study was undertaken with the objectives to explore the rooting and growth response of olive cultivars to air-layering and to find out the appropriate time for air-layerage practice in olives.

Materials and Methods

To determine the "Effect of timing intervals on the rooting response and performance of olive cultivars through air-layerage" the research study was carried out during the year 2014 at Olive Model Farm Sangbhatti, situated in Mardan, Khyber Pakhtunkhwa-Pakistan (Altitude: 375 m; Latitude: 34°16′21.32″N; Longitude: 72°18′06.33″). Mature and healthy plants of the same age and vigor from olive cultivars: Frontoio, Manzanilla, Ottobratica, Pendolino and Picual were selected as mother plants and marked with paint. One year old healthy shoots at all the aspect of the plant canopy were selected and air-layerings were practiced in three plants for each treatment in each cultivar at 15 days intervals, starting from July 1 to September 14, 2014. Six treatments of time intervals, replicated three times for each five cultivars were carried out and 50 air-layerings were practiced per treatment with 4500 layerings in the trial.

The moist medium of silt, saw dust and garden soil (1:1:1 by volume) was placed around the 2-3 cm debarked area of the branch for rooting and wrapped with poly-ethylene film to conserve the moisture and maintain the temperature of root zone. The daughter saplings of each treatment were detached from the mother stock when the roots were developed and grown outside the ball of medium. These were planted in polythene tubes containing media of silt, garden soil and compost in equal proportion by volume; on its own roots for growth and development.

Statistical procedure

The experiment was laid out according to Randomized Complete Block Design (RCBD) with two factors factorial arrangements replicated three times. The data were analyzed according to factorial analysis using Statistix-8.1 software (Statistix_8, 2006). If the data were found significant, these were subjected to Least Significant Difference Test (LSD, $P \leq 0.05$), for mean comparison (Steel et al., 1997).

Results and Discussion

Days to root appearance

The number of days to root appearance from air-layering practice was significantly affected ($P \leq 0.05$) by cultivars, time intervals and their interaction. More number of days to root appearance (71.50) was taken by layering practiced in plants of cultivar Pendolino, the mean of which was significantly varied from rest of the cultivars, followed by number of days to root appearance (61.89) required by layers practiced in plants of cultivar Picual, while less number of days to root appearance (47.94) was taken by the layerings of cultivar Manzanilla.

Table 1: *Days to root appearance, rooting (%), number of roots and root length (cm) of olive cultivars as affected by time of air-layering.*

Olive Cultivars (Cv)	Parameters			
	Days to root appearance	Root percentage (%)	Number of roots	Roots Length (cm)
Frontoio	52.39c	35.61b	3.30b	3.83b
Manzanilla	47.94d	38.89a	4.31a	4.61a
Ottobratica	50.22cd	30.11c	3.49b	3.11c
Pendolino	71.50a	20.78e	2.57d	2.35d
Picual	61.89b	26.89d	2.86c	2.53d
LSD (α = 0.05)	2.457	2.8639	0.2844	0.3790
Timing intervals (T)				
Jul-01	59.47b	19.73e	2.44d	2.49d
Jul-16	56.20c	30.53d	3.56b	3.49c
Jul-31	54.20cd	38.07b	3.87b	3.92b
Aug-15	52.27d	42.40a	4.37a	4.35a
Aug-30	56.13c	34.40c	3.17c	3.25c
Sep-14	62.47a	17.60e	2.43d	2.21d
LSD (α = 0.05)	2.691	3.1422	0.3115	0.4152
Interaction (Cv × T)				
Significance levels	*(Fig 1)	*(Fig 2)	*(Fig 3)	*(Fig 4)

*Mean followed by similar letter(s) in column do not differ significantly from one another. * = Significant at P ≤ 0.05*

The layerings, practiced on 14th September took more number of days to root appearance (62.47) followed by number of days to root appearance (59.47) noted in branches, layered on 1st July, while less number of days to root appearance (52.27) was taken by the layering practiced on 15th August (Table 1).The interaction between olive cultivars and time intervals of air-layering indicated that more days to root appearance were taken by branches of Pendolino when layered on 1st July and less were consumed by the layerings of Manzanilla, practiced on 15th August (Figure 1).

Early root induction and development in Manzanilla might be due to the genetic makeup of the variety which responded efficiently to air layering in comparison with other varieties. The physiological condition of particular stock plant at certain phonological phase has great influence on rooting of layers and appropriate time of layering affected the rooting process in olives. The phenomenon is associated with the activities of cambium tissues, synthesis and movement of photosynthates and other substances that greatly influenced the rooting in air-layerings. Generally, synthesis and accumulation of assimilates and other growth promoters, which initiate rooting and further development influence rooting (Sharma and Srivastav, 2004). The process of development of adventitious roots can be divided in three stages: the initiations of group of meristematic cells i.e. root initials; the differentiation of these initials into root primordia and the development and emergence of new roots including formation of vascular connections with the conducting tissues.

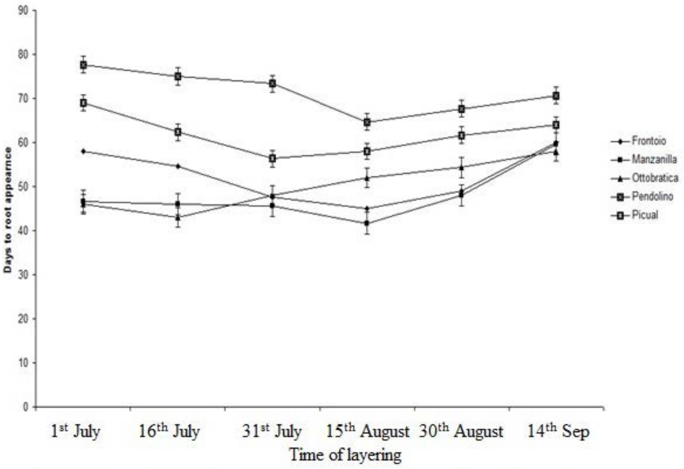

Figure 1: *Days to root appearance of olive cultivars as affected by time of layering.*

In air-layering root initials develop when daughter saplings are still attached to the parent plant. The callus is developed at the upper side of the debarked portion and roots appear from the callus. In olives, the time of air-layering and cultivars greatly influence the number of days required for root development (Rehman et al., 2013), which is also influenced by the size of bark removed (Ahmad et al., 2014). Furthermore,the response of various olive cultivars are different to the number of days required for root development (Butt et al., 1996; Kareem et al., 2013).

Rooting percentage (%)

Significant variations were noted among olive cultivars, time intervals of air-layering and their interaction regarding rooting percentage in air-layered saplings of olive at P ≤ 0.05. High rooting percentage (38.89%) in air layering was achieved by the saplings of cultivar Manzanilla, the mean of which was significantly varied from rest of the cultivars, followed by the rooting percentage (35.61%) recorded in saplings of cultivar Frontoio, while less rooting percentage (20.78%) was obtained in daughter plants of cultivar Pendolino.

The highest rooting percentage (42.40%) was recorded when air-layering was practiced on 15th August, which is statistically varied from rest of the treatment followed by rooting percentage (38.07%) noted in the layers, practiced on 31st July; Whereas lowest rooting percentage (17.06%) was recorded when air-layerings were practiced on 14th September (Table 1).The interaction between cultivars and time intervals of air-layering indicated that high percentage of rooting was attained by the layerings of Manzanilla, layered on 15th August, followed by rooting percentage noted in the daughter saplings of Frontoio at the same time interval, while less rooting percentage recorded for the cultivar Pendolino, layered on 1st July (Figure 2).

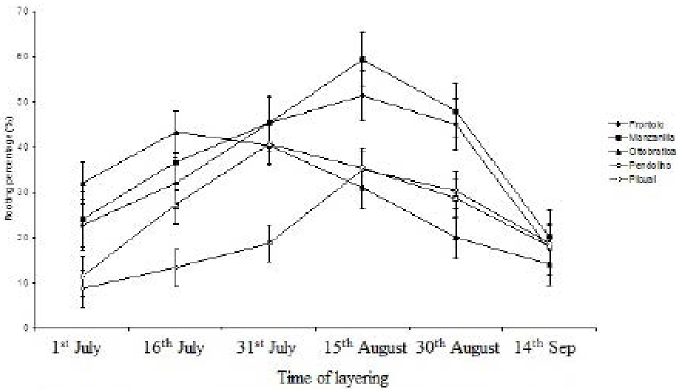

Figure 2: *Rooting percentage (%) of olive cultivars as affected by time of layering.*

Rooting ability depends on many endogenous and exogenous factors such as genetic background, physiological influences, environment (light and temperature), composition of the nutrient medium, age and the ontogenetic phase of the mother plant (Nemeth, 1986). The ability of olive cultivars to generate roots is widely different due to their genetic make-up; some produce roots easily, whereas others are classified as difficult to root cultivars (Sutter and Cohen, 1992; Fabbri et al., 2004). Air-layering at appropriate time and conditions are needed for successful propagation in which rooting percentage is closely associated with the status of the mother stock at certain phase. Rooting percentage of olive cultivars might also be linked to the hormonal activation in the mother plants and optimum environmental conditions which favour rooting in olive. The success in air layering for root development and survival of the layers also greatly depends on various factors such as time of operation, shoot thickness and use of growth regulators (Vyas, 1940). Maximum roots produced from the air-layering, practiced during August (Singh, 1951) and monsoon season when humidity is high (Rehman et al., 2013). Also the rooting percentage and other attributes of the layers are greatly affected when air-layering accomplished in the active growth stage of plants (Shukla and Bajpai, 1974).

Number of roots layering^{-1}

Significant differences were noted among olive cultivars, time intervals of air layering and their interaction regarding number of roots at $P \leq 0.05$. More number of roots (4.31) was produced by the daughter saplings of Manzanilla, which was statistically different from rest of the cultivars followed by 3.49 and 3.30 number of roots generated by layerd branches of Ottobratica and Frontoio respectively and their means were statistically similar, while less number of roots (2.57) was attained in the daughter saplings of Pendolino.

Regarding time intervals of air-layering, more number of roots (4.37) was observed when air-layering was practiced on August 15, followed by the number of roots (3.87), produced from the layering practiced on 31st July, while less number of roots (2.43) was noted in the layering practiced on September 14 (Table 1). As concerned interaction, more roots were induced by the layers of cultivar Manzanilla, practiced on August 15, followed by cultivar Frontoio, layered on the same interval, while the same variety produced less number of roots when air-layered on 1st July and September 14 (Figure 3).

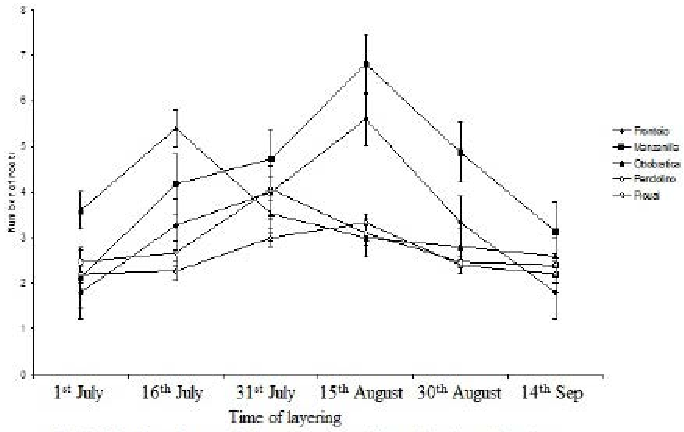

Figure 3: *Number of roots of olive cultivars as affected by time of layering.*

More number of roots in the daughter saplings of Manzanilla might be due the genetic structure of the cultivar, favoured by optimum climatic conditions, efficient utilization of nutrients and hormonal activation during active phase of growth. The time of air-layering in olives have significant effects on number of roots, more roots produced in the air-layering practiced during mid August might be due to the existence of high humidity and more carbohydrates in the active growth phase which moved to the layered portion and initiated more roots induction (Sharma and Srivastav, 2004). Furthermore, early rooting was observed in air-layering, practiced on 15th August, which provided more time for the daughter saplings to produce more roots as compared to other time intervals. The olive cultivars significantly influenced number of roots layering^{-1} (Ahmad et al., 2014); the Frontoio produced more roots as compared to other cultivars (Awan et al., 2003) and monsoon was the proper time for air-layering in olives (Rehman et al., 2013).

Root length (cm)

There were significant variations among the olive cultivars, time intervals of air-layering and their interaction regarding root length in air-layered saplings at $P \leq 0.05$. The lengthy roots (4.61 cm) were produce by daughter saplings of cultivar Manzanilla, followed by root length (3.83 cm) noted in plants of Frontoio, while short root length (2.35 cm) was recorded in plants of Pendolino.

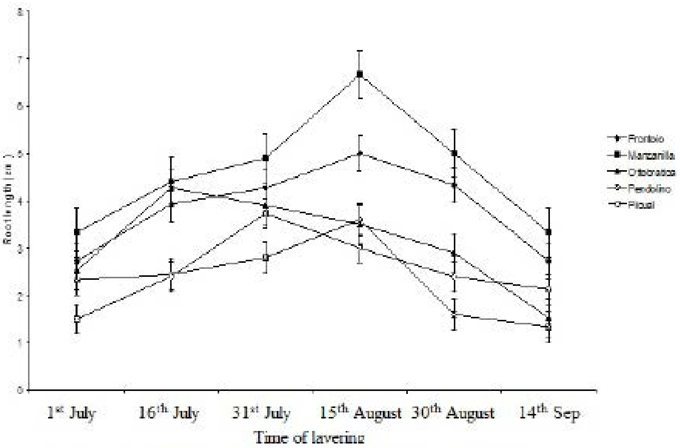

Figure 4: *Root length (cm) of olive cultivars as affected by time of layering.*

Regarding the time of air layering, the maximum root length (4.35 cm) was recorded in the layerings, practiced on 15th August, followed by root length (3.92 cm) generated in saplings layered on 31st July. The saplings of olive cultivars produced short root length (2.21 cm) when layered on 14th September (Table 1). In the interaction long roots were observed in saplings of cultivar Manzanilla, air-layered on August 15, followed by the root length in saplings of the same cultivar, layered on 30th August. The short roots were produced by daughter saplings of cultivar Pendolino, layered on 14th September (Figure 4). The root length in saplings of Manzanilla was more as compared to other cultivars under trail that might be due to the genetic differences among cultivars. The olive cultivar Manzanilla has wide spreading canopy with numerous and lengthier root system and the favourable environmental conditions might be among other reasons which led to the increase in root length. Probably the layers of Manzanilla drew more nutrients and water for photosynthate synthesis that was transported and utilized for roots elongation. Time of layering, also has significant effect on root length due to the efficient utilization of available nutrients present in the mother stock that ultimately transported to the layer portion for development and root elongation. Furthermore, timing is associated with the movement of carbohydrates and other substances controlled by various enzymatic and hormonal activities inside plant body, which has an influential role in root initiation and root length (Sharma and Srivastav, 2004). Early rooting was observed in Manzanilla when layered on 15th August, gave more time to the roots to grow lengthier as compared to the other treatments. Season is responsible for success of layering in woody plants because rooting and root related attributes are enhanced by light duration, presence of sufficient moisture and optimum temperature (Bose et al., 1986). The high percentage of rooting and rooting attributes achieved in *Albizia lucida* and Ficus spp respectively during monsoon (Nautiyal, 2002), similarly August was found the best time for air-layering in litchi regarding rooting success, other rooting attributes and survivability (Dhillon and Mahajan, 2000).

Root thickness (mm)

Significant variations were noted among olive cultivars, time intervals of air-layering and their interaction regarding root thickness of air-layered saplings produced by the studied olive cultivars. Thicker roots (2.34 mm) were induced by the daughter plants of cultivar Pendolino, followed by root thickness (1.99 mm) recorded in plants of Manzanilla, while thinner roots (1.78 mm) were produced by the layers of cultivar Picual.

Regarding time interval of air layering, thicker roots (2.43 mm) were developed by daughter saplings when air-layering was practiced on 15th August, followed by root thickness (2.23 mm) in plants, layered on July 31, while minimum root thickness (1.66 mm) was recorded in the daughter saplings of olive cultivars, layered on 14th September (Table 2).The interaction effects showed that, thicker root were developed by the daughter saplings of Pendolino, layered on 15th August, while thinner roots were noted in plants of cultivar Manzanilla, layered on 14th September (Figure 5).

Table 2: *Root thickness (mm), root weight (g), number of re-sprout and shoot length (cm) of olive cultivars as affected by time of air-layering.*

Olive Cultivars (Cv)	Parameters			
	Root thickness (mm)	Root weight (g)	Number of re-sprouts plant^{-1}	Shoot length (cm)
Frontoio	1.98b	1.46b	2.86b	4.88b
Manzanilla	1.99b	1.77a	3.18a	5.28a
Ottobratica	1.87bc	1.37c	1.84c	4.81b
Pendolino	2.34a	1.29d	2.61b	4.43c
Picual	1.78c	1.34cd	3.17a	4.42c
LSD (α = 0.05)	0.1370	0.0774	0.2782	0.2948
Timing intervals (T)				
Jul-01	1.77de	1.27d	1.93d	4.35c
Jul-16	2.05c	1.43c	2.73c	4.75b
Jul-31	2.23b	1.54b	3.19b	5.43a
Aug-15	2.43a	1.68a	3.67a	5.61a
Aug-30	2.07cd	1.42c	2.95bc	4.31c
Sep-14	1.66e	1.32d	1.92d	4.15c
Jul-01	1.77de	1.27d	1.93d	4.35c
LSD (α = 0.05)	0.1501	0.0847	0.3048	0.3230
Interaction (Cv × T)				
Significance levels	* (Fig 5)	*(Fig 6)	* (Fig 7)	* (Fig 8)

*Mean followed by similar letter(s) in column do not differ significantly from one another. * = Significant at P ≤ 0.05.*

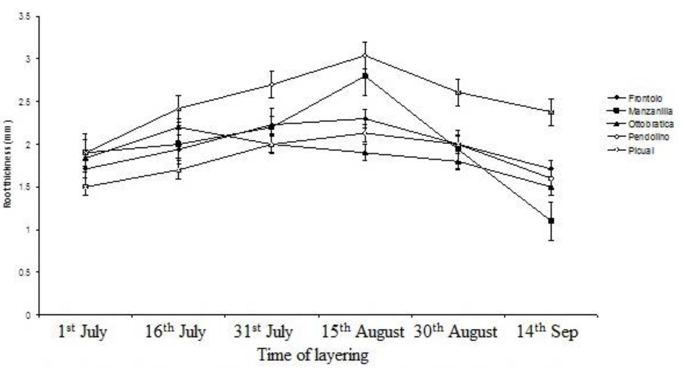

Figure 5: *Root thickness (mm) of olive cultivars as affected by time of layering.*

Different olive cultivars have diverse genetic make-up and respond variously to particular traits. Thicker roots produced by the saplings of Pendolino might be linked to the genetic potential of the variety for horizontal growth of the roots and consumption of more energy on root thickness rather than length and number of roots. The seasonal changes in the rooting response, root weight, length and thickness appears to be regulated by balance of internal translocation of substances including carbohydrates, nitrogenous substance, hormonal growth regulators and co-factors acting synergistically with auxins (Khosla et al., 1982). The olive produced better rooting through air-layering, however the response of different cultivars to rooting through air-layering was significantly varied (Rehman et al., 2013; Ahmad et al., 2014).

Root weight (g)

The data regarding root weight in the daughter saplings produced through air-layering revealed that there were significant variations among cultivars, time intervals of air-layering and their interaction. The daughter saplings of cultivar Manzanilla produced heavy roots (1.77 g) followed by the root weight (1.46 g), generated by the layers of Frontoio and the differences in root weight induced by these cultivars were statistically significant; while light root weight (1.29 g) was attained by the plants of Pendolino.

The maximum root weight (1.68 g) attained by the saplings, layered on 15th August; followed by the root weight (1.54 g) attained by layers when air-layering was practiced on July 31. The air-layered branches produced light root weight (1.27 g) when layered on 1st July (Table 2). In the interaction, heavy root weight was produced by the daughter saplings of Manzanilla, when layerings were practiced on 15th August while roots with less weight were induced by the daughter plants of Pendolino, layered on 1st July and 14th September (Figure 6).

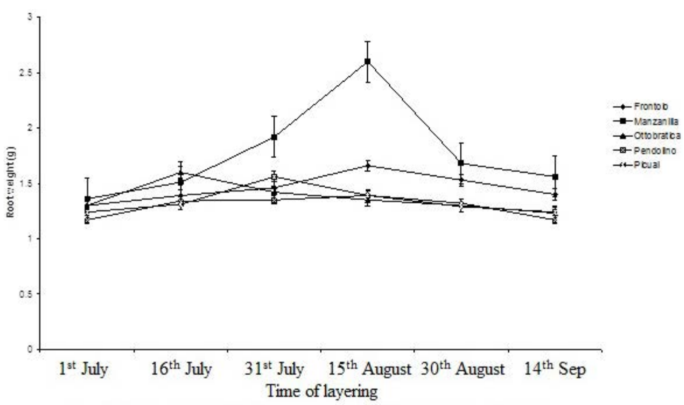

Figure 6: *Root weight (g) of olive cultivars as affected by time of layering.*

Several factors, such as growing phase of mother plants, preferential nutrients uptake, transport, metabolism and gene activation influence the growth and development of plant body. Mother stocks as cultivars have significant effects on root growth and development of olive (Ullah et al., 2012). During plant growth and development the cambium constantly produces cells that differentiate into the phloem towards the outside, and the xylem towards the inside of the trunk. At wounding, an undifferentiated cell mass, or callus, is produced, primarily to heal and seal the wound but when particular hormone concentrations are present; these cells can differentiate into functioning root and adds mass to it.

Number of re-sprouts plant^{-1}

The number of re-sprouts plant^{-1} after detachment and transplantation in the polythene tubes was significantly influenced by cultivars, time intervals of air-layering as well as their interaction. More re-sprouts (3.18) were produced by the daughter saplings of Manzanilla, closely followed by 3.17 re-sprouts generated by the detached rooted layers of cultivar Picual, with statistically similar means. While, less number of re-sprout (1.84) was achieved in the plants of Ottobratica and the variation was statistically significant from the rest of cultivars.

Similarly, more re-sprouts plant^{-1} (3.67) were produced by the detached daughter saplings, layered on 15th August, followed by 3.19 number of re-sprouts in the layerings practiced on 31st July, while few re-sprouts (1.92) were engendered by the detached daughter saplings resulted from the air-layering practiced on 14th September (Table 2). In the interaction more re-sprouts were produced by the detached daughter saplings of Manzanilla layered on 15th August, while few re-sprouts were emerged by the rooted saplings of Ottobratica, layered on 14th September (Figure 7).

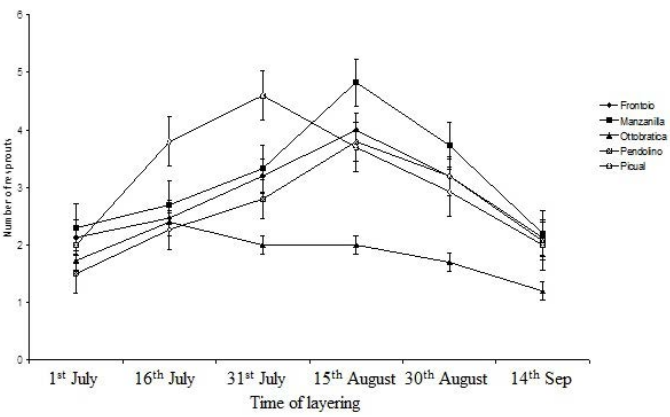

Figure 7: *Number of resprouts of olive cultivars as affected by time of layering.*

The number of re-sprout (fresh shoots) emergence largely depends on the rooting of a plant that provides nutrients to the areal parts. More re-sprouts produced by Manzanilla might be due to genetic potential of the variety having wide canopy, so more shoots would had been produced by it. Furthermore, in layering the stem remained attach with the plant during the process of rooting; nutrients and water were continuously supplied through the intact xylem layer and better nutrition and water supply helped in prolific rooting which led to the increased number of shoots (Sharma and Srivastav, 2004). The constant supply and better utilization of available resources by Manzanilla, the cultivar led to increase in number of shoots. Time interval of air layering also had a significant influence on number of shoots which can be linked with conducive environmental conditions for the layers to produce vigorous root system that lead to the generation of more shoots.

Shoot length (cm)

The shoot length of the detached daughter saplings was significantly influenced by the olive cultivars, time intervals of air-layering and their interaction $P \leq 0.05$. Long shoots (5.28 cm) were developed by the re-sprouts of cultivar Manzanilla after detachment and transplantation, followed by the shoot length (4.88 cm) produced by the daughter saplings of Frontoio. The short length of shoot (4.42 cm) was noted in the plants of cultivar Picual.

Regarding time intervals, long shoots (5.61cm) were produced in the detached daughter saplings, layered on 15th August, followed by shoot length (5.43 cm) attained by the re-sprouts, layered on 31st July, while short shoots length (4.15 cm) was developed by the detached daughter saplings resulted from the air-layering of 14th September (Table 2). The interaction effect showed that, lengthy shoots were recorded in saplings of cultivar Frontoio layered on August 15, while short shoot length was produced by the daughter plants of cultivar Ottobratica, when air-layering was practiced on 14th September (Figure 8).

Vegetative growth of air layered saplings largely depends on the rooting response of the cultivars. The lengthy shoots produced by the saplings of cultivar Manzanilla might be due to the effective uptake and utilization of nutrients, conducive environmental conditions for growth and development and other genetically controlled factors. Probably the inner strength of Manzanilla which led to increment in the shoot length played an effective role. Similarly the time of air layering also had an influential effect on the shoot length because air-layering practiced at appropriate time induced profuse rooting in olive due to enzymatic and hormonal activitation at active growth stage, favoured by optimum environmental conditions. The well established root system probably enhanced the uptake of nutrients; those might had been utilized for growth and development of plant organ.

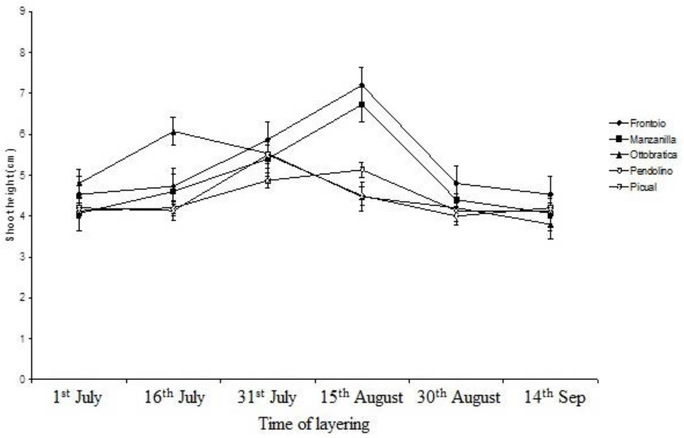

Figure 8: *Shoot height (cm) of olive cultivars as affected by time of layering.*

Conclusions and Recommendations

It is concluded from the results of the present study that optimum time for air-layering in olive is mid August (15th August) in terms of rooting percentage, number of root, root length, weight, number of resprout and shoot length. The olive cultivars: Frontoio, Manzanilla and Pendolino are recommended to be air-layered during mid August. Ottobratica and Picual are preferred to be layered during mid and late July respectively, under the local conditions of Sangbhatti, Mardan.

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Author's contributions

Riaz Alam conducted the research, collected data, did statistical analysis and wrote the paper. Muhammad Sajid supervised the study and provided technical input at every step.

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