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Rooting Ability of Some Olive Genotypes by Sub-Terminal Cuttings

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Abstract: This study was carried out during two successive seasons (2014 and 2015) on the sub-terminal cuttings of fifteen of evaluated olive genotypes that selected from the Genetic improvement of olive in the experimental orchard of Horticulture research institute in Egypt, to identify the genotypes ability of rooting formation. The fifteen genotypes were grouped at four groups (Manzanillo, Aggizi, Arpequina and Koroneiki) and the main brand used as a mother. The cuttings were treated with Indol-3-butric acid at 4000 ppm (as a commercially used in olive) and planted under mist condition at four months (January, April, July and October) for studying the physiological, chemical constituents and anatomical examination of the cuttings and the relationship between them that leading to formation of roots. The obtained results could be summarized as follows: A wide variation in rooting ability among the evaluated olive genotypes cuttings were detected. The cuttings of genotype (125) indicated the highest rooting percentage, average number of roots percentage, leaf and stem carbohydrates content, while the cuttings of genotype (86) was the lowest during April collection date in both studied seasons. While, the cuttings of genotype (86) gave the highest leaf and stem nitrogen content, in both seasons during the October collection date. Anatomical structure of the sub-terminal cuttings of genotypes (125,75,43 and 100)as easy - to-root and (86, 73, 107 and 53) hard – to- root reveled that, in-hard to root genotypes the lowest forming adventitious root may be correlated with the density of continuity of the sclerenchyma Ring, forming mechanical barriers to emergence of newly formed roots. On the other side, in the easy to root genotypes, the best forming of roots exhibited at genotype (125) following by genotypes (75, 43 and 100).The adventitious root primordial consequently show more developing characteristic, its vascular system develops and contacts with the main xylem vessels and primordial gradually penetrates the cortex. Moreover, low thickness of the cortex cell layer possibly allow to the emerge of newly formed roots.

Key words: Olive *(Olea europaea)* · Rooting cuttings · Genotypes · Histological characteristics

INTRODUCTION

Olive *(Olea europaea)* is one of the most important fruit species that thrive successfully on many arid and semi-arid lands and play an important role in the economy of such areas, due to an increasing interest in olive oil for human consumption. The domesticated olives originated based on decades of selection from the natural forest around the Mediterranean basin being one of the oldest cultivated tree crops [1]. New olive orchards are being planted outside the Mediterranean, calling for an effort to identify the genotypes best adapted to the new conditions however, some olive cultivars remain difficult to propagate, which significantly reduced the capacity to use the full genetic diversity of the species. Studying the rooting ability of cutting has become a critical topic, which implies fundamental research on anatomy, physiology, biochemistry and genetics of the adventitious root formation process [2]. In Egypt, olive cultivation increased considerably during the last few decades and there are many newly introduced genotypes that resulted from the olive improvement program in horticultural research institute [3]. Evaluated and studying rooting ability of these genotypes are very important to determine a different rooting behavior of these genotypes before cultivated in newly reclaimed areas. Among the vegetative propagation methods of olive cultivars stem cuttings are considered the most simple and economical method of olive propagation. Commercially olives are propagated by stem cutting, which favors the speedy nursery production [4]. Cutting propagation relies on the ability of the cuttings to form adventitious roots [5].

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While some cultivars are easily propagated by this technique, others are difficult-to-root and this poses a challenge for their preservation and commercialization [6, 7]. Rooting aptitude of the different olive cuttings depends on both intrinsic and extrinsic factors, i.e., cultivars, rooting media and time of planting [8]. The mist propagation system is one of the best ways that has a high propagation coefficient and produces a powerful and resistant seedling to diseases. Indole butyric acid (IBA) the most commonly used to promote the rooting in cuttings of a wide range of plant species. [9]. In olive cuttings, Indole butyric acid applications are a limiting successful factor for rooting in olive cuttings. But poor rooted cultivars may not respond well to exogenous IBA [10, 11]. Insensitivity to applied IBA in olive cuttings was explained by the differences in metabolism and transport of IBA [12]. With a view towards the future, propagation of the selected superior genotypes is very important to identify the genotypes ability of rooting formation before cultivated it in the reclaimed area. The present experiment aimed to study the rooting ability of sub-terminal cuttings of fifteen of olive superior genotypes, the external factors, endogenous chemical content and histological characteristics that affect on the ability to root of each genotype adaptable under Egypt conditions. Meantime, the genotypes under this study have been previously evaluated in terms of vegetative, productivity, fruit characteristics and oil quality, which achieved present agronomical behaviors and quality of the oil, that make them interesting for the use in new olive orchards, also for the production of oils with a strong yet greatly diverse typicality [13].

MATERIALS AND METHODS

Experimental Site and Plant Material: This study was carried out during 2014 and 2015seasons, on the cuttings of fifteen evaluated olive genotypes derived through Genetic improvement of Olive (CFFC) IOOC, project 001, which performed the best agronomical characteristics (productivity and oils) at the experimental orchard of Horticulture Research Institute at Giza, Egypt. Trees were about 15- year-old in a good physiological condition and received normal agriculture procedures. Therefore, the fifteen genotypes were grouped at four groups and the main brand used as a mother (receptor ♀). Furthermore, the groups were divided as follow: Manzanillo group which included of five genotypes, Aggizi group which included four genotypes, Arpequina group which included four genotypes and Koroneiki group which included two genotypes arranged as shown in Table (1).

Table 1: Sources of genotypes according to the project map of Olive improvement program

Tree No. of the Genotypes project map	Groups	Derived from
86	Manzanillo ♀	open pollination
88	Manzanillo ♀	open pollination
93	Manzanillo ♀	open pollination
95	Manzanillo ♀	open pollination
100	Manzanillo ♀	open pollination
32	Aggizi ♀	open pollination
73	Aggizi ♀	Aggize x Coronaki
75	Aggizi ♀	Aggize x Picual
151	Aggizi ♀	Aggize x Manzanillo
107	Arpequina ♀	Arpequina open
125	Arpequina ♀	Arpequina x Toffahi
129	Arpequina ♀	Arpequina x Aggize
144	Arpequina ♀	Arpequina x Hamed
53	Koroneiki ♀	open pollination
43	Koroneiki v	Koroneiki x Hamed

Cutting Collection and Preparation: Sub-terminal cuttings were taken from moderator vigorous shoots of current growth. The cuttings were at 12-15 cm long one-year shoots/season. Two pairs of terminal leaves on each cutting were retained and the basal cut was made just below the node. Cuttings of fifteen genotypes were planting at four dates, in the first of January, April, July and October during each season.

Rhizogenic Treatments: The genotypes cuttings (sub terminal cuttings) were performed by dipping about (2-3 cm) basal part of cuttings in the Indole butyric acid solution (IBA) at 4000 ppm for 5 seconds according to Kurd *et al.* [11], after that treated with benlate solution (1g /L) as fungicide. Then planted to a depth of 5 cm in a plastic box filled with a mixture of vermiculite and sand (1:2 volume), each box contains about 120 cuttings (three replicates). The planted cuttings were put in a rooting bench provided with basal heating (substrate temperature 22-24°C) and with mist system to get periodically wet the cuttings avoiding their dehydration (air humidity about 90-95%) in seran shaded house. The cuttings were taken 70 days after the rooting treatments to evaluate the following:

Rooting Ability of Cuttings: The percentage of rooting was calculated as the number of rooted cuttings with respect to the total number of cuttings, the average number of roots/rooted cutting, were counted and the average root length /rooted cutting was measured for each genotype.

Endogenous Constituents of Cuttings Tissues: Samples were taken from leaves and shoots of cuttings that planted in four dates, at the first of (January, April, July, October) during the two seasons and dried to determine the following:

- Total carbohydrates content was estimated according to Masuko *et al*. [14].
- Total Nitrogen Percentage was determined by using the modified microkjeldal method as described by Bremner *et al*. [15].
- C/N ratio was calculated by divided the percentage of total carbohydrates by that the total of nitrogen.

Anatomical Studies: Eight Samples of olive genotypes from the basal portion (3 cm) of cutting of four groups which achieved the lowest and highest rooting percentage was taken to study the formation of adventurous root. Materials were killed and fixed in FAA solution until sectioning and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax melting point 56-58°C as described by Johansen [16]. Sections were cut on a rotary microtome at a thickness of 20-25 microns were stained with safranine and light green before mounting in Canada balsam [16, 17]. Slides were examined microscopically and photomicrography.

Experiment Design and Statistical Analysis: The experiment was laid out; using Randomized Complete Block Design (RCBD) with two factors, factorial arrangement, four dates of cutting propagation and fifteen of olive genotypes. Analysis of various was computerized by MSTATC software program. Data were tabulated and statically analyzed according to Snedecor and Cochran [18]. Means were compared using Duncan's Multiple Range test [19].

RESULTS AND DISCUSSIONS

Rooting Ability of Sub Terminal Olive Cuttings: The capability of olive genotypes cuttings that treated with IBA at (4000 ppm) for rooting forming was significantly affected by the genotypes (G), Dates of cutting collection (D) and the interaction between (G × D) as follow:

Rooting Percentage: The means of rooting percentage of fifteen olive genotypes that cleared in Table (2) illustrated the significant differences in the rooting percentages of studied genotypes during different planting dates. Highest rooting percentage was obtained from genotype (125), while genotype (86) gave lowest rooting percentage in both seasons. Meanwhile, the rooting percentage reached to the highest values during April and October planting dates in the first season, while it was in April at the second one. Moreover, the cuttings that planted in January exhibit the lowest rooting percentage in both seasons. Additionally, rooting percentage responded significantly to the interaction among (GxD), the highest value was obtained from genotypes 125 and 75 during April and October planting dates in the first season, while, in the second season, the highest values were obtained from genotypes125 and 75 during April and from genotype 125 during October.

Table 2: Effect of planting dates on rooting percentage of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	7.28 z	17.62xy	11.04 z	20.70w-y	14.16 M	8.11z	15.30w-y	12.97xy	19.83u-w	14.05 J
88	11.75 z	28.34s-u	18.59w-y	28.91s-u	21.90 L	14.02xy	26.66p-s	21.17t-v	27.74o-r	22.40 I
93	22.55v-x	39.37n-p	31.39r-t	43.59 l-o	34.23 I	20.49t-v	38.70lm	29.26o-q	45.12i-k	33.39 G
95	23.96u-w	42.64l-o	33.48q-s	42.16m-o	35.55 H	22.43s-u	40.93j-l	35.61mn	42.32j-l	35.32 F
100	30.75r-t	60.99ij	47.11k-m	70.60d-f	52.36 F	29.06o-q	62.35ef	49.86h-i	69.97cd	52.81 E
32	37.79o-q	67.33f-h	50.30k	64.62g-j	55.01 E	40.56j-m	70.04cd	48.43hi	65.22d-f	56.06CD
73	21.46w-y	40.59n-p	29.84st	48.18kl	35.02 H	23.61r-u	40.03k-m	31.77no	45.51ij	35.23 F
75	31.29r-t	84.46a	60.22j	83.80ab	64.94 B	30.63op	86.70 a	61.40ef	48.11hi	56.71 C
151	40.57n-p	73.33de	61.88h-j	68.79e-g	61.14 C	38.47lm	75.17b	60.33 f	67.33 d	60.33 B
107	9.21 z	37.72o-q	16.92 y	36.20p-r	25.01 K	11.83yz	38.51lm	17.22v-x	40.20k-m	26.94 H
125	41.00n-p	81.75ab	62.80h-j	85.62 a	67.79 A	41.05j-l	84.82 a	60.76f	84.92 a	67.89 A
129	30.78r-t	78.71bc	50.71k	67.41f-h	56.90 D	29.81o-q	75.77 b	53.18g	65.84de	56.15CD
144	27.40t-v	64.79g-j	42.27m-o	66.63f-i	50.27 G	30.49op	73.79bc	41.35j-l	65.21d-f	52.71 E
53	11.56z	45.07k-n	23.87u-w	45.11k-n	31.40 J	15.10w-y	45.48ij	25.14q-t	45.47ij	32.80 G
43	31.46r-t	74.91cd	49.79 k	61.66h-j	54.46 E	32.76no	77.18 b	50.96gh	60.57 f	55.37 D
Mean	25.25 C	55.84 A	39.35 B	55.60 A	---	25.89 D	56.76 A	39.96 C	52.89 B	---

Means having similar letters in the same row or column were not significantly different at 0.05 probability

Table 3: Effect of planting dates on rooting number of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	3.00s	4.33o-r	3.33rs	4.33o-r	3.75 I	3.67q	5.00m-o	4.00pq	5.00m-o	4.42 I
88	3.67q-s	8.33gh	7.00i-k	5.33m-o	6.08DE	4.67n-p	7.67e-g	7.33f-h	6.00j-l	6.42EF
93	4.00p-s	6.67j-l	5.67l-n	5.33m-o	5.42FG	5.00m-o	6.33i-k	5.33l-n	6.00j-l	5.67GH
95	4.00p-s	6.67j-l	5.33m-o	5.00m-p	5.25GH	5.00m-o	7.00g-i	5.00m-o	5.00m-o	5.50H
100	6.00k-m	10.33cd	8.00g-i	7.33h-j	7.92C	4.00pq	10.00c	7.33f-h	7.67e-g	7.25D
32	4.67n-q	10.67bc	8.00g-i	6.67j-l	7.50C	5.33l-n	10.33c	8.00ef	7.00g-i	7.67C
73	4.00p-s	8.00g-i	6.67j-l	5.33m-o	6.00DE	4.33o-q	8.00ef	7.00g-i	5.00m-o	6.08F
75	4.00p-s	8.00g-i	6.00k-m	5.33m-o	5.83EF	4.67n-p	9.00d	6.33i-k	5.67k-m	6.42EF
151	4.33o-r	11.33b	9.67de	8.67fg	8.50B	4.33o-q	12.00b	9.00d	8.33de	8.42B
107	5.00m-p	8.33gh	5.33m-o	5.33m-o	6.00DE	5.67 k-m	8.00ef	5.67 k-m	5.00m-o	6.08F
125	6.67j-l	12.33a	9.33ef	8.00g-i	9.08A	6.33i-k	13.00a	9.00d	7.67e-g	9.00 A
129	3.33rs	6.33j-l	5.00m-p	4.67n-q	4.83H	5.33l-n	6.67h-j	5.33l-n	6.67h-j	6.00FG
144	5.33m-o	9.33ef	8.33gh	7.00i-k	7.50C	5.67k-m	9.00d	8.00ef	7.67e-g	7.58CD
53	6.00k-m	4.33o-r	8.00g-i	7.33h-j	6.42D	6.67h-j	5.00n-o	7.33f-h	7.00g-i	6.50E
43	5.00m-p	7.00i-k	7.00i-k	6.33j-l	6.33DE	5.33l-n	7.67e-g	6.33i-k	7.33f-h	6.67E
Mean	4.60D	8.13A	6.84B	6.13C	---	5.07D	8.31A	6.73B	6.47C	---

Means having similar letters in the same row or column were not significantly different at 0.05 probability

Table 4: Effect of planting dates on rooting length (cm) of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	8.00e-n	11.83 a	7.93f-n	10.60a-c	9.59B	9.07e-g	11.20 a	7.13i-k	10.50 b	9.48A
88	5.10s-y	7.17j-q	5.17r-x	6.67l-t	6.00G	4.43t-w	7.80 h	4.83r-u	6.13l-n	5.80F
93	5.57q-w	6.37l-u	4.73u-z	5.80o-v	5.62G	6.13l-n	7.10 jk	5.27p-r	5.20p-s	5.93F
95	7.23i-q	9.90b-e	7.67g-p	9.67b-f	8.62D	6.30l-n	8.97 fg	6.20l-n	8.93fg	7.60D
100	3.20yz	4.67u-z	3.00z	4.07v-z	3.73 I	4.27u-w	5.70n-p	4.07vw	5.00q-t	4.76H
32	7.57h-p	9.50b-g	7.00k-r	9.43b-h	8.38D	7.17i-k	8.60 g	6.47lm	8.77fg	7.75D
73	8.30d-l	12.00a	9.50b-g	11.23ab	10.26A	7.53h-j	11.33 a	9.03e-g	10.17bc	9.52A
75	6.80l-s	10.33a-c	8.97c-j	9.96b-d	9.02C	7.70hi	10.67 b	8.60g	9.27ef	9.06B
151	6.10m-u	9.00c-j	5.53q-x	8.75c-k	7.35E	5.37p-r	8.00 h	5.20p-s	7.87 h	6.61E
107	5.73p-v	7.70g-o	5.40q-x	7.30i-q	6.53F	5.07q-s	6.40 lm	5.03q-t	6.53lm	5.76F
125	3.10yz	4.67u-z	3.90v-z	4.10v-z	3.94HI	3.97w	6.00m-o	4.87q-t	4.00 w	4.71H
129	4.83t-z	7.33i-q	4.60u-z	6.07n-u	5.71G	5.47o-q	6.23l-n	5.30p-r	6.10l-n	5.78F
144	8.03e-m	11.17ab	7.07j-r	10.23a-c	9.13C	7.80h	9.87cd	6.70kl	9.57de	8.48C
53	3.60x-z	5.47q-x	3.73w-z	4.43u-z	4.31H	5.00q-t	6.40 lm	4.60s-v	5.30p-r	5.33G
43	6.93k-r	10.27a-c	8.00e-n	9.13c-i	8.58D	6.03m-o	9.100e-g	7.40h-j	8.67fg	7.80D
Mean	6.00C	8.49A	6.15C	7.83B	---	6.09C	8.22A	6.05C	7.47B	---

Means having similar letters in the same row or column were not significantly different at 0.05 probability

The Average Rooting Number per Rooted Cutting: Data presented in Table (3) show marked differences among the fifteen olive genotypes on number of roots per rooted cuttings during different collections dates. The greatest number of roots was observed for genotype (125). On the contrary, genotype (88) exhibited the lowest one in both seasons. As for the effect of the date of planting on the average rooting number, April planting date achieved the maximizing root number per cuttings, while January achieved the minimizing root number in both seasons of study. Also, significant interaction between (G x D) was observed in genotype 125 during two studied seasons.

The Average Root Length (cm) per Rooted Cutting: Data in Table (4) showed that, root length (cm) per rooted cuttings of tested olive genotypes exhibited a variability in the length of root during the collection dates, the highest increase produced from the planted cutting in the first of April, while January and July produced the lowest one. Furthermore, the rooted cuttings of genotype (73) gave significantly the longest roots. On the other hand, the genotype (100) produced significantly the shortest roots in the first season. While, in the second season, each of genotypes (73 & 86) gave the longest root, although the genotypes (125 & 100) gave the shortest one. Moreover, there were a significant response

Table 5: Effect of planting dates on leaf carbohydrates content of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	10.20yz	13.86p-v	14.00o-v	11.66v-z	12.43 J	10.75u	14.00no	15.01m	11.98st	12.94 J
88	11.84v-z	16.23j-o	17.13f-m	13.40q-w	14.65 H	12.00st	16.43 l	17.76ef	13.67no	14.96 G
93	12.11v-y	19.55a-e	16.78g-n	13.88p-v	15.58 F	11.77 t	19.92ab	17.06h-k	14.00no	15.69 E
95	13.29r-w	19.84a-c	18.19a-j	15.76k-p	16.77BC	13.82no	20.03ab	17.29f-i	15.22m	16.59 B
100	10.98x-z	18.45a-j	16.59h-n	13.07s-x	14.77 H	10.92u	17.57e-h	16.80i-l	12.75qr	14.51 H
32	12.08v-y	18.41a-j	16.85g-n	14.60n-u	15.48 F	12.07st	18.75d	16.68j-l	13.99no	15.37 F
73	13.36q-w	19.62a-d	17.79b-k	15.49k-r	16.57 C	13.09pq	20.00ab	17.21g-j	14.95m	16.31 C
75	11.89v-z	20.36 a	17.44d-l	14.67n-u	16.09 E	12.37rs	20.07ab	17.62e-g	14.12n	16.05 D
151	12.91t-x	19.93ab	18.74a-h	15.62k-q	16.80 B	13.02pq	19.58bc	19.00d	15.29m	16.72 B
107	9.77 z	15.62k-q	13.23r-x	11.24w-z	12.46 J	10.00v	15.35 m	13.66no	11.13u	12.54 K
125	13.66p-v	20.25a	18.59a-i	16.34i-n	17.21 A	13.80no	20.27a	18.04e	16.28l	17.10 A
129	13.16s-x	19.22a-f	17.64c-l	15.36l-s	16.34 D	13.04pq	19.09cd	16.73i-l	15.07m	15.98 D
144	11.09w-z	17.31e-m	15.12m-t	13.17s-x	14.17 I	10.95u	17.11g-j	14.90m	13.51op	14.12 I
53	11.83v-z	18.51a-j	16.59h-n	13.87p-v	15.20 G	12.00st	18.05e	16.51kl	13.92no	15.12 G
43	12.58u-x	18.97a-g	17.59c-l	14.71n-u	15.96 E	12.26r-t	19.15cd	17.03h-k	14.99m	15.86DE
Mean	12.05D	18.41A	16.82B	14.19C		12.12D	18.36A	16.75B	14.06C	

Means having similar letters in the same row or column were not significantly different at 0.05 probability

of root length per cuttings in both seasons to the interaction appearance in the cuttings of genotype (86,73and 144) that prepared in the first of April and October in partnership with genotype (43) in April the first season. While in the second season, the highest values were obtained by genotypes (86 and73) in April.

The ability of sub terminal olive cuttings for rooting formation was significantly different for the studied genotypes of olive under different collection dates, the food material in the cuttings may be utilized as source for root formation because at this stage they had no root for uptake of the nutrients [20, 21]. Moreover, period of cuttings collection significantly affected the rooting aptitude of cultivars and rooting strongly depended on interaction with cultivar, time of collection and type of cuttings [22, 23]. Also, there was correlation between ability of rooting and average number of roots for olive cultivars that was reported by [24, 9, 21]. Despite some discordant results obtained that, time of the year where cuttings are taken is important to get a maximum rooting, in a general, seasonal trend for rooting percentage with a maximum in summer and minimum during autumn and winter [**25].**

Endogenous Constituents of Sub Terminal Cuttings Tissues

Total Carbohydrates Percentage: Data presents in Tables (5 & 6) indicate that total carbohydrates content in leaves and stems differed among genotypes due to different collections dates.

A-Leaf Carbohydrates Content: Data in Table (5) illustrate that leaves of genotype (125) recorded the highest significant percentage of carbohydrates content in comparison to leaves of genotypes (86 that achieved the lowest carbohydrates content in both studied seasons with participation of genotype (107) in the first season. In general, leaves carbohydrates content of tested olive genotypes was significantly higher during the first of April than other dates in both studied seasons. Meanwhile, there was a convergence of leaves carbohydrate content responded to the interaction in the April.

Stem Carbohydrates Content: Total carbohydrates were obtained from Genotypes (125) and (75) in the first season Moreover, the genotype (125) achieved the highest carbohydrate content in the second one. Whereas, genotype (86) comprised the lowest content as shown in Table (6). Moreover, stem cuttings of different olive genotypes that planted in April gave the highest total carbohydrates content. On the other hand, January date gave the lowest in both studied seasons. As regarding to interaction effect, there was a convergence of leaves carbohydrate content among genotypes in April during two studied seasons. Moreover, stem of genotypeso

Table 6: Effect of planting dates on stem carbohydrate content of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	20.86yz	26.85j-m	24.63qr	21.91wx	23.56 J	21.53w-y	26.12i-m	25.75k-o	22.33vw	23.93 K
88	21.11x-z	28.78f-h	27.00j-l	25.29pq	25.54 G	20.38z	29.06cd	27.57fg	23.75s-u	25.19GH
93	21.90wx	30.22a-c	26.83j-m	25.89n-p	26.21EF	20.80yz	30.42ab	25.82j-o	24.25q-t	25.33 G
95	24.66qr	29.27d-g	28.12hi	26.11m-p	27.04 C	24.00r-t	28.02ef	27.22f-h	25.16m-q	26.10EF
100	22.52vw	30.07a-d	27.35i-k	25.34pq	26.32DE	21.15yz	29.00cd	27.00g-i	22.96uv	25.03 H
32	21.63xy	29.75b-e	26.55k-n	24.53qr	25.61 G	22.11v-x	29.05cd	26.88g-i	26.19i-l	26.06 F
73	20.76z	30.66a	29.00e-g	25.27pq	26.42 D	25.00n-q	29.92bc	29.82bc	25.03n-q	27.44 B
75	24.55qr	30.56ab	30.18a-c	25.83n-p	27.78 A	21.26x-z	31.04a	30.33ab	26.09i-m	27.18 C
151	22.82uv	30.12a-b	29.91a-d	26.13l-p	27.25 B	21.34x-z	30.19ab	28.48de	25.00n-q	26.25 E
107	20.99yz	24.49qr	24.16rs	23.32tu	23.24 K	20.83yz	25.84j-n	25.83j-o	24.83o-r	24.33 J
125	24.68qr	30.67a	29.48c-f	26.85j-m	27.92 A	24.00r-t	31.07a	28.80se	27.25f-h	27.78 A
129	2406r-t	28.70f-h	26.28l-o	25.31pq	26.09 F	23.55tu	27.51fg	24.93n-r	25.00n-q	25.25 G
144	21.38x-z	25.85n-p	26.68k-n	23.67st	24.39 H	20.59yz	26.73g-j	27.31f-h	24.68p-s	24.83 I
53	20.85yz	26.25l-o	24.61qr	24.49qr	24.05 I	21.30x-z	26.83g-i	25.40l-p	25.25l-p	24.70 I
43	23.43s-u	27.59ij	28.53gh	25.66op	26.30DE	24.57p-s	28.05ef	27.00g-i	26.40h-k	26.51 D
Mean	22.41D	28.66A	27.29B	25.04C	---	22.16D	28.59A	27.21B	24.95C	---

Means having similar letters in the same row or column were not significantly different at 0.05 probability

Table 7: Effect of planting dates on leaf nitrogen content of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	1.250b-f	1.260 b-e	1.200c-k	1.727 a	1.359A	1.280ab	1.060w-z	1.250a-e	1.280ab	1.217A
88	1.113k-r	1.050qr	1.087m-r	1.120k-q	1.092G	1.103q-x	1.050yz	1.093s-y	1.113p-v	1.090 I
93	1.117k-r	1.030r	1.097l-r	1.183d-l	1.107FG	1.110p-w	1.030z	1.113p-v	1.190g-m	1.111 H
95	1.147h-p	1.050qr	1.127j-q	1.270b-d	1.148DE	1.160j-p	1.050yz	1.110p-w	1.270a-c	1.148 E
100	1.183d-l	1.60p-r	1.170e-m	1.217b-j	1.158C-E	1.180h-n	1.057x-z	1.170i-o	1.217d-i	1.156 D
32	1.160f-n	1.067o-r	1.153g-o	1.183d-l	1.141D-F	1.153k-q	1.063v-z	1.137m-t	1.170i-o	1.131 G
73	1.200c-k	1.080n-r	1.183d-l	1.233b-h	1.174CD	1.190g-m	1.087t-y	1.170i-o	1.227c-h	1.168 C
75	1.227b-i	1.077n-r	1.213b-j	1.250b-f	1.192BC	1.100r-y	1.087t-y	1.210d-j	1.260a-d	1.164 C
151	1.127j-q	1.030r	1.147h-p	1.190d-k	1.123E-G	1.120o-u	1.033z	1.140m-s	1.180h-n	1.118 H
107	1.163f-n	1.040qr	1.177e-l	1.223b-i	1.151DE	1.130n-t	1.060w-z	1.180h-n	1.203e-k	1.143EF
125	1.147h-p	1.077n-r	1.140i-p	1.177e-l	1.135D-F	1.223c-h	1.053x-z	1.187g-m	1.270a-c	1.183 B
129	1.247b-f	1.077n-r	1.240b-g	1.290b	1.213B	1.247a-f	1.070u-z	1.243a-e	1.290a	1.215 A
144	1.197c-k	1.043qr	1.190d-k	1.230b-i	1.165C-E	1.223c-h	1.033z	1.197f-l	1.233b-g	1.172 C
53	1.283bc	1.050qr	1.257b-e	1.300b	1.222B	1.150k-r	1.050yz	1.160j-p	1.183g-m	1.136FG
43	1.143h-p	1.063p-r	1.143h-p	1.177e-l	1.132EF	1.130n-t	1.077u-z	1.147l-r	1.180h-n	1.133 G
Mean	1.180 B	1.101 C	1.168 B	1.220 A	---	1.167 B	1.057 C	1.168 B	1.218 A	---

Means having similar letters in the same row or column were not significantly different at 0.05 probability

(75) achieved the highest carbohydrates content in July in both studied season with participation of genotype (151) in the first season.

Total Nitrogen Percentage

A-Leaf Nitrogen Content: As regard in results that presented in Table (7) it could be noticed that genotype (86) in both seasons with participation of genotype (129) in the second season exhibited high nitrogen content. Whereas, the genotype (88) was the lowest level in both seasons. On the contrary, cuttings that prepared in October gave the highest level of total nitrogen in two studied seasons. In additions, the genotype (86) planted in the first of October was a superior in the first season as the effect of the interaction, while, a convergence during different planting dates in the leaves nitrogen content was observed in the second season.

Stem Nitrogen Content: Data in Table (8) showed that nitrogen percentage in stems of different olive genotypes were fluctuated due to difference planting dates. Stems of genotype (86) in both seasons with the participation of genotypes (73 and 53) in the second season possessed the highest significant level of total nitrogen comparing

Table 8: Effect of planting dates on stem nitrogen content of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	0.730bc	0.650jk	0.700ef	0.750a	0.708A	0.730b-d	0.620m-o	0.720c-e	0.740a-c	0.703 A
88	0.700ef	0.600op	0.660ij	0.690fg	0.663F	0.710d-f	0.620m-o	0.650j-l	0.700e-g	0.670EF
93	0.713c-e	0.620mn	0.70hi	0.700ef	0.676DE	0.730b-d	0.610no	0.680g-i	0.690f-h	0.678DE
95	0.700cd	0.620mn	0.660ij	0.720cd	0.680CD	0.710d-f	0.610no	0.650j-l	0.700e-g	0.668 E
100	0.710de	0.630lm	0.680gh	0.730bc	0.688BC	0.710d-f	0.630l-n	0.690f-h	0.710d-f	0.685CD
32	0.730bc	0.620mn	0.670hi	0.710de	0.683CD	0.730b-d	0.650j-l	0.660i-k	0.700e-g	0.670EF
73	0.720cd	0.613m-o	0.700ef	0.740ab	0.693B	0.700e-g	0.640k-m	0.710d-f	0.750ab	0.700AB
75	0.667h-j	0.590 p	0.640kl	0.620mn	0.629G	0.710d-f	0.620m-o	0.700e-g	0.730 b-d	0.690 C
151	0.730bc	0.610no	0.650jk	0.690fg	0.670EF	0.710d-f	0.630l-n	0.640k-m	0.700 e-g	0.670EF
107	0.683f-h	0.620mn	0.650jk	0.713c-e	0.667F	0.667ij	0.630l-n	0.670 hi	0.630 b-d	0.674 E
125	0.690fg	0.610no	0.690fg	0.730bc	0.680CD	0.700e-g	0.600 o	0.700 e-g	0.740 k-m	0.688 C
129	0.700ef	0.620mn	0.680gh	0.720cd	0.680CD	0.690f-h	0.600 o	0.690 f-h	0.730 b-d	0.678DE
144	0.710de	0.620mn	0.700ef	0.750a	0.695B	0.700e-g	0.620 m-o	0.690 f-h	0.760 a	0.693BC
53	0.710de	0.620mn	0.667h-j	0.740ab	0.684CD	0.730b-d	0.630 l-n	0.710 d-f	0.750 ab	0.700AB
43	0.720cd	0.610no	0.700ef	0.720cd	0.695B	0.680g-i	0.610 no	0.627 mn	0.730 b-d	0.639 F
Mean	0.709 B	0.617 D	0.674 C	0.717 A	---	0.708 B	0.621 D	0.679 C	0.717 A	---

Means having similar letters in the same row or column were not significantly different at 0.05 probability

Table 9: Effect of planting dates on leaf C/N ratio of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	10.93p-y	12.97j-p	15.49d-i	12.80k-r	13.05FG	9.58u	18.07cd	13.63j	11.71n-q	13.24 F
88	10.64r-y	15.46d-i	15.76d-i	11.97n-v	13.46DE	10.88rs	15.65g	16.25f	12.28mn	13.76 E
93	10.85p-y	18.99ab	15.30e-i	11.73o-w	14.22 C	10.60st	19.34a	15.32g	11.76n-q	14.25 C
95	11.62o-w	18.90ab	16.15c-h	12.41l-u	14.77 B	11.91m-p	19.08a	15.57g	11.98m-p	14.64 B
100	9.28x-z	17.41a-d	14.18h-m	10.74q-y	12.90 G	9.25uv	16.63f	14.36i	10.48st	12.68 G
32	10.42t-z	17.27b-e	14.61f-k	12.34m-u	13.66 D	10.47st	17.63de	14.68hi	11.95m-p	13.68 E
73	11.14o-x	18.17a-c	15.03f-j	12.56k-t	14.23 C	11.00rs	18.41c	14.71hi	12.20m-o	14.08CD
75	9.74w-z	18.92ab	14.39g-m	11.74o-w	13.69D	11.24qr	18.47bc	14.56i	11.21qr	13.87DE
151	8.40 o-w	15.02f-j	12.28o-x	9.19x-z	11.22HI	11.63o-q	18.95ab	16.67f	12.96kl	15.05 A
107	8.89yz	12.88k-q	11.25h-m	9.91v-z	10.73 I	8.85v	14.48i	11.58pq	9.25uv	11.04 J
125	11.40z	19.35a	16.34c-g	13.13 j-o	15.162 A	11.28qr	19.24a	15.21gh	12.82kl	14.64 B
129	10.56s-y	17.85a-c	14.23m-u	11.91n-v	13.64 D	10.46st	17.84d	13.35jk	11.68o-q	13.33 F
144	9.27x-z	16.59c-f	12.70k-s	10.71q-y	12.32 H	8.95v	16.56f	12.45lm	10.95rs	12.23 H
53	9.80v-z	18.07- a c	14.00i-n	11.32o-x	13.30EF	9.43u	13.00kl	13.09k	10.76 t	11.44 I
43	10.36u-z	17.40a-d	14.51f-l	11.78o-w	13.52DE	10.51st	17.19e	14.23i	11.16n-q	13.40 F
Mean	10.22 D	17.00 A	14.42 B	11.63 C	---	10.40 D	17.37 A	14.38 B	11.54 C	---

Means having similar letters in the same row or column were not significantly different at 0.05 probability

with other genotypes. In the contrary, stems of tested genotypes recorded the highest significant level of nitrogen content during October comparing to April which achieved the lowest value. Furthermore, the genotypes (86, 73,144 and 53) that planted in October gave the highest leaf nitrogen content as the effect of interaction in both studied seasons.

C/N Ratio: Data in Tables (9 &10) indicate that C|N ratio in leaves and stems of fifteen olive genotypes under study was significantly affected by the different collection dates.

Leaves C/N Ratio: Data in Table (9) indicate that leaves of genotype (125) recorded the highest C/N ratio in the first season. Whereas, the genotype of (151) was the highest value in the second season. Moreover, the lowest C|N ratio in the leaves was obtained from the genotype (107) in both seasons. In contrast, leaves of cuttings that prepared in April had the highest leaf C/N ratio comparing to other collection dates. As the effect of interaction, a convergence between genotyped that planted in April in the first season. While, the genotypes 93, 95, 75,151 and 125 achieved the highest leaf C/N ratio.

Table 10: Effect of planting dates on stem C/N ratio of sub terminal olive genotypes cuttings during 2014 & 2015 seasons

	Planting Dates									
Genotypes	January	April	July	October	Mean	January	April	July	October	Mean
	2014 season					2015 season				
86	31.29v-z	45.52b-f	38.50i-q	35.36n-v	37.67 D	31.66q-t	42.83e-i	41.09h-j	34.96m-p	37.63CD
88	30.16x-z	47.97a-c	40.91g-l	36.66l-t	38.92 C	28.71u	46.88cd	42.61f-i	34.04n-q	38.06CD
93	30.70w-z	48.75a-c	40.05h-m	37.07k-t	39.14 C	28.52u	49.88ab	37.99kl	35.15m-o	37.89CD
95	34.29p-x	49.48ab	44.68c-g	37.31j-s	41.44 A	34.40n-p	46.70cd	38.89jk	33.65n-r	38.41C
100	30.47x-z	47.22a-d	39.04h-o	33.64r-y	37.59 D	29.89tu	46.10cd	39.14jk	32.36p-s	36.85DE
32	30.85w-z	48.50a-c	40.83g-l	35.68m-v	38.97 C	29.56tu	44.88d-f	40.93ij	37.41k-m	38.20C
73	28.45z	47.18a-d	41.44f-k	33.78r-y	37.71 D	35.83l-n	46.79cd	42.00g-i	33.48n-r	39.52 B
75	34.10q-x	49.98a	43.11d-h	34.97o-w	40.54AB	29.94tu	50.10ab	43.49e-h	35.76l-n	39.82 B
151	31.26v-z	49.42ab	46.02a-e	37.87j-r	41.14A	30.06s-u	48.00bc	44.51d-g	35.72l-n	39.57 B
107	30.72w-z	39.51h-n	37.28j-s	32.74t-z	35.06E	31.28r-t	41.03h-j	38.61jk	34.03n-q	36.24EF
125	34.29p-x	49.48ab	44.68c-g	37.31j-s	41.06 A	33.81n-q	50.94a	44.47d-g	39.07jk	40.37 A
129	34.37p-x	46.30a-e	38.67i-p	35.17n-w	38.63 C	34.14n-q	46.05cd	36.14l-n	34.26n-p	37.65CD
144	30.12x-z	41.70f-j	38.15i-r	31.65u-z	35.41 E	29.42tu	43.14e-i	39.59jk	32.54o-r	36.17EF
53	29.37yz	42.34e-i	37.01k-t	33.10s-y	35.46 E	29.22tu	42.59f-i	35.78l-n	34.61n-p	35.55 F
43	32.55t-z	45.22b-f	40.85g-l	34.22p-x	38.21CD	33.69n-r	45.27de	37.51k-m	35.68l-n	38.04CD
Mean	31.63D	46.47A	40.49B	35.00C	----	31.34 D	46.08 A	40.18 B	34.85 C	----

Means having similar letters in the same row or column were not significantly different at 0.05 probability

Stem C/N Ratio: Stems of genotypes (95, 75, 151 and 125) in the first season and (125) in the second season possessed the highest significant content of C/N ratio in comparison of other genotypes. In contrast, April preparation date induced the highest significant content as comparing with other dates in both seasons. According to the interaction, no clear difference between most of genotypes during April in the first season. While, each of genotypes (93, 75 and 125) in the second season achieved the highest stem C|N ratio.

Many investigations proved that high levels in Carbohydrates were favorable for rooting of cuttings and there was correlation between high C/N ration content and rooting percentage as [26, 27]. Moreover, carbohydrates in the olive stem cuttings generally have a positive effect on rooting ability of the cuttings [28, 29]. In addition, a balance among endogenous stimulatory and inhibitory factors, as well as, nutrients and carbohydrates that led to promote rooting of olive cuttings [30, 23]. Nitrogen levels appeared to be an important predictor for rooting potential of stem. It is believed that the function of nitrogen in root formation in cuttings is related to its role in the synthesis of nucleic acids and proteins. The effects of nitrogen contents on root initiation and development in stem cuttings depend on several factors such as carbohydrate availability, C/N ratio and interactions between endogenous hormones [31, 32, 7]. C/N ratio is important at the rooting ability, the researchers Denaxa *et al.* [29] and Mahmood *et al.* [26] reported that (total carbohydrate) / N ratios were more closely correlated with rooting, due to roles of carbohydrates as the source of energy and their requirements for macromolecules synthesis during the root formation process.

Anatomical Study of Rooting Formation: Stem cross sections of easy and hard- to-root cuttings of olive genotypes in four groups were compared to study the anatomical differences among them those effects on the formation of adventitious roots. A microscopically study reveled that, root initiation in olive genotypes under study was found to originate from the cambium zone, which the cells were divided to form the root initial and root primordial (Fig. 1 and 2). In hard- to- root genotypes (86, 73, 107 and 53) that presented in Fig (1), the lowest forming adventitious root may be correlated with the density of continuity of the sclerenchyma Ring, forming mechanical barriers that inhabit the organization of new cells to form root primordial which led to inhibit forming of adventitious root. Furthermore, adventurous root of genotype (73,107 and 53), were more than genotype (86) due to slightly interrupted of the sclerenchyma ring, that helping for emerging a number of adventitious roots than 86. On the other side, in the easy to root genotypes, that presented in Fig. (2). It could be noticed from illustrate of these genotypes that cell division was observed only on the cambium zone and gave rise to different layers of tissues which formed the root initials and root primordial. The sclerenchyma ring did not remain intact and dissolving during the rooting period as regard to

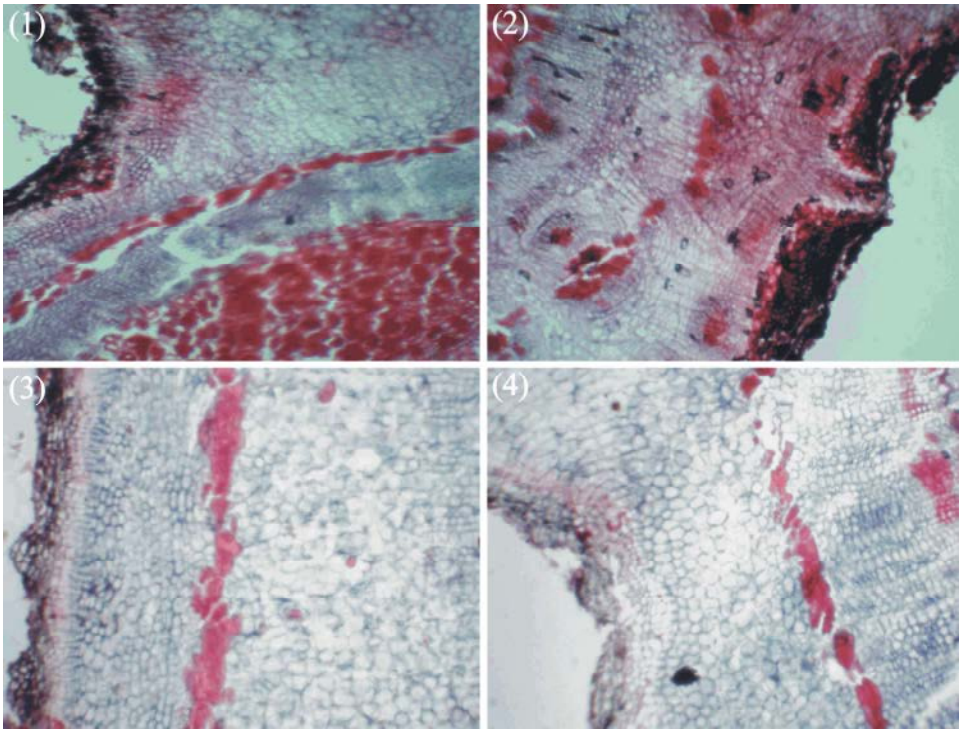

Fig 1: Transverse sections of hard to root cuttings of olive showing formation of roots where:
1- Genotype 86 2- Genotype 73 3- Genotype 107 4- Genotype 53

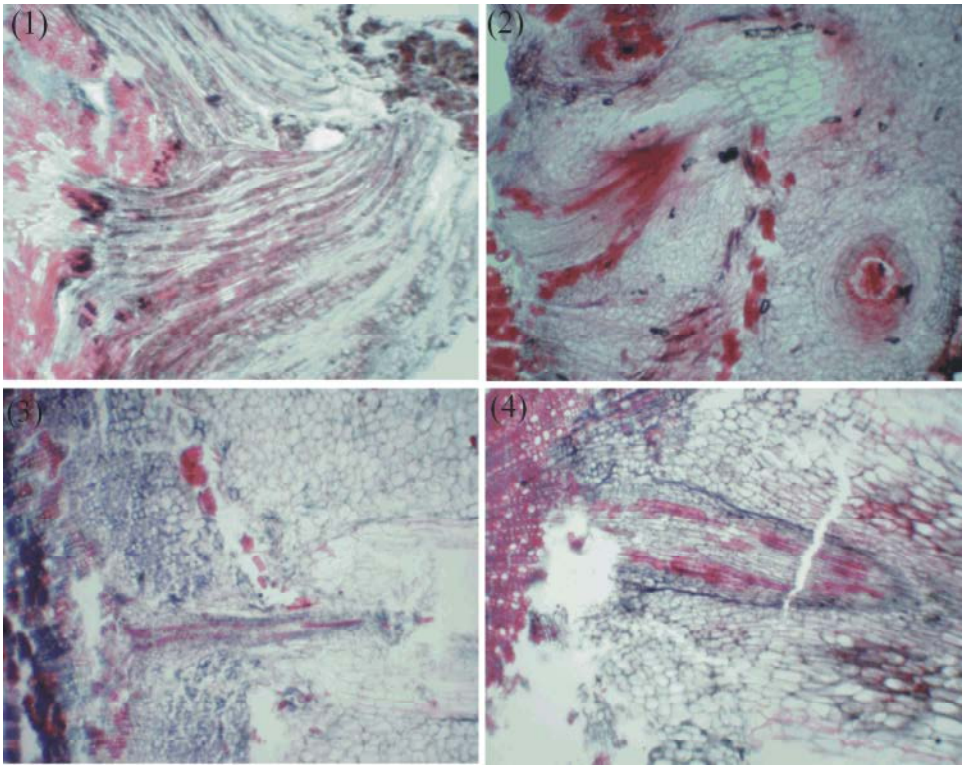

Fig 2: Transverse sections of easy to root cuttings of olive showing formation of roots where:
1- Genotype 125 2- Genotype 75 3- Genotype 45 4- Genotype 100

genotype (125). The adventitious roots primordial consequently show more developing characteristic, its vascular system develops and contacts with the main xylem vessels and primordial gradually penetrates the cortex. Moreover, low thickness of the cortex cell layer possibly allows emerging of newly formed roots. Merely the same results was obtained for genotypes (75,100 and 43) which also share for forming roots but less than genotype (125) due to the presence of sclerenchyma ring but its more interrupted which allow to emerge of newly formed roots. These findings supported by Several anatomical studies which have suggested a correlation between difficulty in rooting and the presence of continuous sclerenchyma layer that might act as a physiological barrier to adventitious root initiation or a mechanical barrier to root emergence [33, 34, 27]. Moreover, low rooting percentage might be due the presence of continuous sheath of sclerenchyma, forming mechanical barrier to emergence of newly formed rootlets [35, 20, 36, 37].

Generally, it may be concluded that, the genotype (125) achieved the highest root formation, while the genotype (86) achieved the low formation of roots. The hard- to- root in the cuttings of genotype '86' could be attributed to decrease in carbohydrates content and the low in C/N ratio that important for rooting formation. Moreover, low rooting percentage might be due the presence of continuous sheath of chelernchyma, forming mechanical barrier to emerge of newly formed roots decrease the appearance of initiate adventurous root. Thus, the genotype '86' description as a difficult-to-root. In addition, the genotype (125) achieved the high formation of roots, due to the increase in carbohydrates content and the high in C/N ratio. Moreover, the high formation of roots may be mentioned that, the sclerenchyma ring did not remain intact and dissolving during the rooting period that leads for forming adventitious root rapidly. So the genotype '125' description as easy-to-root.

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