

# Investigation on rooting ability of twenty olive cultivars from Southern Italy

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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**Abstract:** The effects of two different types of auxins (660 ppm alpha-naphthaleneacetic acid - NAA - in liquid solution or 750 ppm alpha-naphthaleneacetamide - NAD - dispersed in a talcum powder) and cuttings from three different portions of the shoots (basal, middle and apical) on the rooting ability of twenty autochthonous olive cultivars were investigated in two growing seasons (spring and autumn). The results showed that the autochthonous olive cultivars of the Campania Region are characterized by a wide variability in the potential rhizogenic ability. The two periods of cutting collection (March and September) significantly affected the rooting aptitude of the cultivars, indicating that in some cultivars the cuttings collected in autumn had a higher rooting rate than those collected in spring. The effects of NAA and NAD on rooting strongly depended on interaction with the cultivar, time of collection (autumn or spring) and type of cuttings (basal, medium or apical). Among the twenty cultivars tested, we found only eight cultivars with a satisfactory rooting ability after hormonal applications (Ortolana, Racioppella, Tenacella, Tonda, Biancolilla, Carpellesse, Cornia and Pisciotana). In general, the apical and the median portions of the shoots gave the best rooting results.

## 1. Introduction

Among the vegetative propagation methods of olive cultivars (*Olea europaea* L.) the use of semi-hardwood cuttings is the most common, since rootings are easy to prepare and the requirement in special equipment is negligible and cheap (Cimato, 1999; Ismaili *et al.*, 2011). This is why in the Mediterranean basin olive is mainly propagated by cuttings, a propagation method that relies on the ability of the cuttings to form adventitious roots (Fabbri *et al.*, 2004). While some cultivars are easily propagated by this technique, others are difficult-to-root and this poses a challenge for their preservation and commercialization (Hartmann and Kester, 1975; Hartmann *et al.*, 1990; Carfi *et al.*, 1994; Porfírio *et al.*, 2016). Rooting aptitude of the different olive cuttings depends on both intrinsic and extrinsic factors (Wiesman and Lavee, 1994; Hechmi *et al.*,

2013), such as the genotype (Avidan and Lavee, 1978; Fabbri *et al.*, 2004; Chiancone *et al.*, 2011), the age of the mother tree, the timing of cutting collection as well as the type of cuttings (Fontanazza and Jacoboni, 1975; Del Rio *et al.*, 1991; Khabou and Trigui, 1999). Taking this background into consideration, the main aim of this study was to assess the rooting ability of olive cuttings obtained from twenty autochthonous olive cultivars of the Campania Region, by using different combinations of auxin treatments (NAA and NAD), times of collection of cuttings (spring and autumn) and portions of shoots to prepare the cuttings (basal, middle or apical). The cultivars considered in this study have been previously evaluated in terms of vegetative-productive characteristics and oil quality, and some of them present agronomical behaviors and quality of the oils which make them interesting for the use in new olive orchards, also for the production of oils with a strong yet greatly diverse typicality (Di Vaio *et al.*, 2013). This makes very important to know their intrinsic ability to root and the best combination of factors (hormones, time of cutting collection and portion of shoots to use) to utilize in order to obtain the best rooting results from each cultivar.

## 2. Materials and Methods

### *Experimental site and plant material*

The trial was carried out in 2014/2015 at the Experimental Station "Improsta" of the Campania Region, located in Battipaglia (40°37' 00" N lat, 15°03' 23" E long, 72 m above sea level) Sele Plain (Salerno, South Italy). The average annual rainfall and the average minimum and maximum temperature of the area were 988 mm, 10.9°C and 21.0°C, respectively.

The experimental station hosts a germplasm collection orchard, established in 2001, which includes all the main autochthonous olive cultivars of the Region. Trees were trained at central leader and spaced 6 × 3 m.

Among the available germplasm, 20 cultivars were selected for their economic importance and potential to be used in new orchards considering their good agronomical characteristics (productivity and resistance to biotic and abiotic stresses) and high quality/typicality of the oils, since some of them also allow the production of certified oils under the Protected Designation of Origin. The cultivars were grouped according to their origin (main province of

cultivation). Therefore, the selected olive cultivars were four from the province of Avellino ('Ogliarola campana', 'Ravece', 'Ritonnella' and 'Ruveia'), five from the province of Benevento ('Femminella', 'Ortice', 'Ortolana', 'Pampagliosa' and 'Racioppella'), four from the province of Caserta ('Asprinia', 'Caiazzana', 'Tonda' and 'Tenacella') and seven from the province of Salerno ('Biancolilla', 'Carpellese', 'Cornia', 'Oliva Bianca', 'Pisciottana', 'Rotondella' and 'Salella') (Di Vaio *et al.*, 2013).

### *Cutting collection and preparation*

The cuttings were collected in the autumn (end of September) and in the next spring (second decade of March). For each cultivar, three trees were selected by following homogeneity criteria for developmental stage and productivity. The semi-hardwood cuttings were obtained in autumn (A) and spring (S) from 30 cm long one-year shoots/season. Each shoot was divided in three different portions (basal, middle and apical) (180 cuttings/cultivar/season). Cuttings were 10 cm long and presented 4 nodes and 2 pairs of terminal leaves.

### *Rhizogenic treatments*

For each combination of cultivar, collection time, portion of shoot and hormonal treatment three groups of ten cuttings each were used. The trial was conducted under mist system in cold greenhouse conditions. Two different commercial formulations of auxins, alpha-naphthaleneacetic acid (NAA) in liquid formulation (hydroalcoholic solution at 30% alcohol) at the concentration of 660 ppm (T1) and alpha-naphthaleneacetamide (NAD) at the concentration of 750 ppm (T2) in talcum powder formulation (0.075/100 w.w.) were compared to untreated control (T0). These concentrations were used considering the good results obtained with NAA 500-1000 ppm in other cultivars (Denaxa *et al.*, 2011). The T1 treatment was performed by dipping 2 cm basal part of cuttings in the hydroalcoholic solution for 5 seconds, whereas the T2 treatment by dipping 2 cm basal part of cuttings in distilled water first and then in the powder. The T0 (control) was obtained by dipping 2 cm basal part of cuttings in distilled water for 5 seconds. Cuttings were then placed in perlite filled rooting benches 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%).

### *Rooting sampling and score*

Semi-hardwood cuttings were evaluated 70 days after the rooting treatments and each cutting was

scored for the rooting rate, the number of roots/cutting (primary and secondary roots), and the length of roots/cutting (the length of the different roots of each cutting was summed). The percentage of rooting was calculated as the number of rooted cuttings with respect to the total number of cuttings per treatment.

*Statistical analysis*

All data were statistically analyzed by three-way analysis of variance (ANOVA) using the SPSS 13 software package (SPSS 13.0 for Windows; SPSS Inc., Chicago, IL). Whenever the two-way interaction was significant, a one-way ANOVA was performed. To separate treatment means for each measured parameter, Duncan’s multiple range test was performed at a significance level of  $P \leq 0.05$ .

**3. Results and Discussion**

Adventitious rooting process in cuttings is still to be unraveled under the genetic point of view, however, as referred in studies on Italian and international olive cultivars (Fontanazza and Baldoni, 1989; Chiancone *et al.*, 2011), the capability of cuttings of forming adventitious roots is mainly affected by the cultivar. Among the wide number of local and worldwide grown cultivars, the main part of them displays a poor aptitude for rooting of cuttings, whereas few cultivars are known to be well rooting cultivars. A dataset of the International Olive Council (IOC, 2005) reported the results of a screening on rooting rate of 426 cultivars: 59 cultivars showed an average rooting rate of 1.5%, 213 cultivars reached an average rate of 21.3%, whereas the rooting rate recorded in 86 cultivars was approximately 54% and only 68 (=16% of the total) showed a rate of rooting higher than 70%.

In the present study, rooting rate of the cuttings was significantly affected by the cultivar (C), by the interaction between the cultivar and the time of sampling (C × TS) and by the interaction between the hormonal treatment and the time of sampling (T × TS) (Table 1). The highest percentage of rooted cuttings, over the rooting treatment and the time of sampling, was recorded in cv. Ortolana (66%), followed by cv. Racioppella (54.6%) and cv. Biancolilla (51%) (Fig. 1). On the contrary, cv. Ogliarola campana, cv. Ortice and cv. Salella showed the lowest rooting rate (14.9%, 15.8% and 11.2%, respectively).

Timing of cutting collection, according to the literature, is the second pivotal element, following the cultivar, to be concerned about, since very relevant

differences in the success of rooting process may depend on this factor (Hartmann and Loreti, 1965). Thus, the definition of the most suitable season for cutting collection from mother plants has been a critical item for researchers dealing with fruit trees species, although the fine tuning of protocols ready to use in the nurseries is still lacking, especially if single cultivars are considered. The C × TS interaction highlighted that the cultivars considered in this study responded differentially to the time of cutting sampling (Fig. 2). For instance, among the three cultivars that showed the highest rooting rate, cv. Ortolana and cv. Racioppella reached higher percentages of rooting when the cuttings were collected during spring season, whereas the rooting rate of cv. Biancolilla was significantly increased by using the cuttings collected in autumn (Fig. 2). On the contrary, the cultivars that showed the lowest rooting rate (cvs. Ogliarola campana, Ortice and Salella) did not

Table 1 - Analysis of variance for cultivars, hormonal rooting treatments, time of sampling and their interactions on rooting rate of olive cuttings

Source of variance	Rooting rate (%)
Cultivar (C)	***
Hormonal treatment (T)	NS
Time of sampling (TS)	NS
C × T	NS
C × TS	***
T × TS	*
C × T × TS	NS

NS= Non significant; \*, \*\*, \*\*\*= significant at  $P \leq 0.05$ ,  $\leq 0.01$ ,  $0.001$ , respectively.

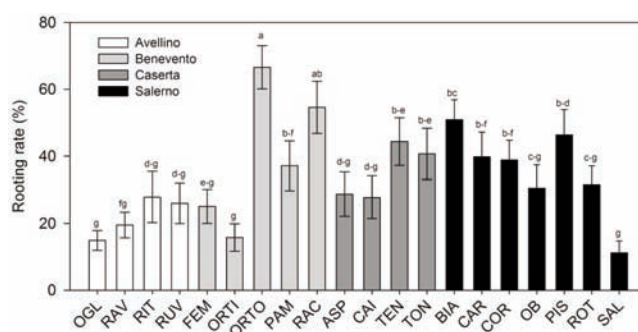


Fig. 1 - Average rooting rate (mean ± standard error) of semi-hardwood cuttings of the olive cultivars of the Campania Region. Bars with the same colour correspond to cultivars of the same province. Abbreviations of the names of the cultivars: OGL = Ogliarola campana, RAV = Ravece, RIT = Ritonnella, RUV = Ruveia, FEM = Femminella, ORTI = Ortice, ORTO = Ortolana, PAM = Pampagliosa, RAC = Racioppella, ASP = Asprinia, CAI = Caiazzana, TEN = Tenacella, TON = Tonda, BIA = Biancolilla, CAR = Carpelliese, COR = Cornia, OB = Oliva Bianca, PIS = Pisciotana, ROT = Rotondella, SAL = Salella. Means followed by different letters are significantly different for  $P \leq 0.001$ .

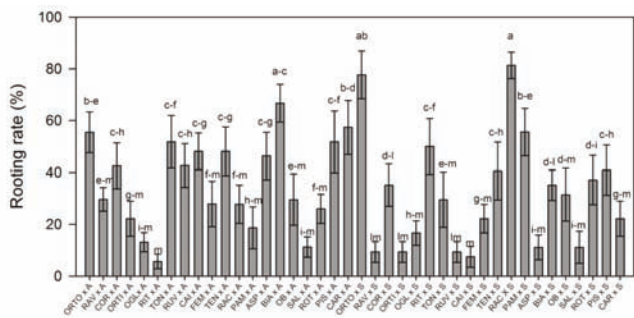


Fig. 2 - Average rooting rate (mean ± standard error) of semi-hardwood cuttings as affected by the interaction between the cultivar and the time of sampling. A= autumn; S= spring. Abbreviations of the names of the cultivars: OGL = Ogliarola campana, RAV = Ravece, RIT = Ritonnella, RUV = Ruveia, FEM = Femminella, ORTI = Ortice, ORTO = Ortolana, PAM = Pampagliosa, RAC = Racioppella, ASP = Asprinia, CAI = Caiazzana, TEN = Tenacella, TON = Tonda, BIA = Biancolilla, CAR = Carpellese, COR = Cornia, OB = Oliva Bianca, PIS = Pisciotana, ROT = Rotondella, SAL = Salella. Means followed by different letters are significantly different for P<0.001.

perform differently comparing the two times of sampling (Fig. 2).

The overall influence of hormonal treatments (NAA or NAD) and time of sampling (T × TS) on cutting rooting rate indicated that the effect of treatments was significantly different on the cuttings collected in spring (Fig. 3). In particular, the lowest rooting rate was observed on T0 cuttings collected in spring (Fig. 3).

On the base of cutting rooting rate, the International Olive Council classified the cultivars in four ranks: 0-5%; 5-40%; 40-70% and 70-100% (IOC, 2005). According to this classification, the twenty cultivars tested in the present experiment resulted to be distributed in three different groups: twelve between 5 and 40% ('Ogliarola campana', 'Ravece', 'Ritonnella', 'Ruveia', 'Femminella', 'Ortice', 'Pampagliosa',

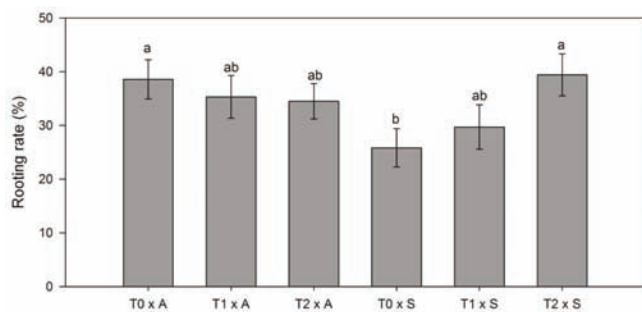


Fig. 3 - Average rooting rate (mean ± standard error) of semi-hardwood cuttings as affected by the interaction between the hormonal rhizogenic treatments (T0= control - no hormonal treatments; T1= NAA treatment; T2= NAD treatment) and the time of sampling. A= autumn; S= spring. Means followed by different letters are significantly different for P ≤ 0.05.

'Asprinia', 'Caiazzana', 'Oliva Bianca', 'Rotondella', 'Salella'); seven between 40 and 70% ('Racioppella', 'Tenacella', 'Tonda', 'Biancolilla', 'Carpellese', 'Cornia' and 'Pisciottana'); one between 70 and 100% ('Ortolana').

As far as root number and length are concerned, the effects of cultivar, hormonal treatments and portion of the shoot and their first level interactions were all significant (Table 2). The twenty cultivars of olive exhibited a high variability in the number and length of roots produced both in autumn and spring experiments. The root number and length were increased by the rhizogenic treatments. Generally, both the liquid and powdery formulations used for the rooting stimulation of the cuttings were effective in improving rooting emission and growth. With regard to the number of roots, our findings indicated that the liquid treatment with 660 ppm NAA was the most effective, showing, with respect to the control, an average increase of about 266% in cuttings from the basal, median and apical portions of the shoots. The same treatment was particularly effective in stimulating the length growth of the roots, with an average increase of 236% compared to the control. Furthermore, the root number and length of cuttings collected in autumn and spring were significantly affected by the interaction between the cultivar (C) and hormonal rooting treatment (T) (Table 2). For instance, the average root number in autumn cuttings was 2.7 roots per cutting, and the application of the rhizogenic treatment T2, compared to T0, induced a significant increase of the number of roots in nine cultivars: Ortolana (+245%), Cornia (+169%), Tonda (+160%), Ruveia (+165%), Tenacella (+192%), Asprinia (+312%), Biancolilla (+320%), Pisciotana

Table 2 - Analysis of variance for cultivars, hormonal rooting treatments, shoot portion and their interactions on number and length of roots of olive cuttings obtained in autumn or spring

Source of variance	Root number		Root length (cm/cutting)	
	Autumn	Spring	Autumn	Spring
Cultivar (C)	***	***	***	***
Hormonal treatment (T)	***	***	***	***
Portion (P)	***	**	***	***
C × T	**	***	***	**
C × P	*	***	*	***
T × P	*	NS	NS	*
C × T × P	NS	***	NS	*

NS= Non significant; \*, \*\*, \*\*\*= significant at P ≤ 0.05, ≤ 0.01, 0.001, respectively.

(+610 %) and Carpellese (+236 %) (Fig. 4A).

The average root length in autumn cuttings was 4.2 cm per cutting, and, similarly to the root number, it was also increased by the rhizogenic treatment T2

in ten cultivars: Ortolana, Ravece, Cornia, Tonda, Ruveia, Tenacella, Asprinia, Biancolilla and Pisciotana (Fig. 4 B). A similar pattern was observed in the spring cuttings (Fig. 5 A, B), even though the

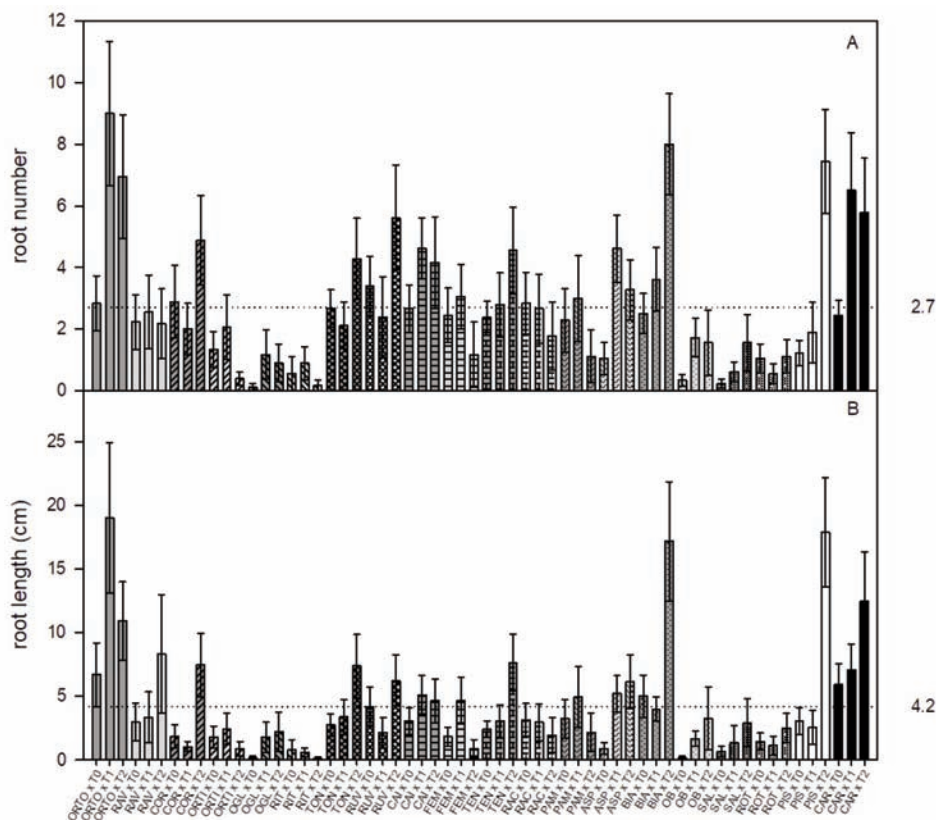


Fig. 4 - Effects of the interaction of the cultivars and the hormonal rhizogenic treatment (T0= Control - no hormonal treatments; T1= NAA treatment; T2= NAD treatment) on root number/cutting (A) and root length/cutting (B) of semi-hardwood cuttings collected in autumn. The dotted line shows the average value of all cultivars under observation. Abbreviations of the names of the cultivars: OGL = Ogliarola campana, RAV = Ravece, RIT = Ritonnella, RUV = Ruveia, FEM = Femminella, ORTI = Ortime, ORTO = Ortolana, PAM = Pampagliosa, RAC = Racioppella, ASP = Asprinia, CAI = Caiazzana, TEN = Tenacella, TON = Tonda, BIA = Biancolilla, CAR = Carpellese, COR = Cornia, OB = Oliva Bianca, PIS = Pisciotana, ROT = Rotondella, SAL = Salella.

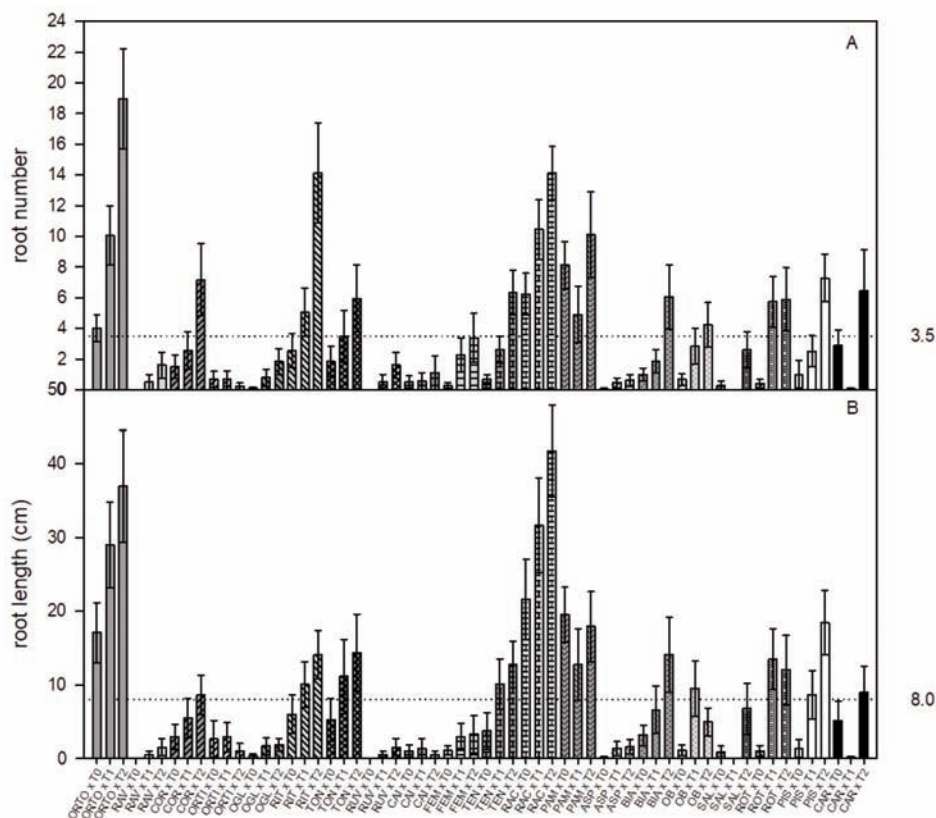


Fig. 5 - Effects of the interaction of the cultivars and the hormonal rhizogenic treatment (T0= Control - no hormonal treatments; T1= NAA treatment; T2= NAD treatment) on root number/cutting (A) and root length/cutting (B) of semi-hardwood cuttings collected in spring. The dotted line shows the average value of all cultivars under observation. Abbreviations of the names of the cultivars: OGL = Ogliarola campana, RAV = Ravece, RIT = Ritonnella, RUV = Ruveia, FEM = Femminella, ORTI = Ortime, ORTO = Ortolana, PAM = Pampagliosa, RAC = Racioppella, ASP = Asprinia, CAI = Caiazzana, TEN = Tenacella, TON = Tonda, BIA = Biancolilla, CAR = Carpellese, COR = Cornia, OB = Oliva Bianca, PIS = Pisciotana, ROT = Rotondella, SAL = Salella.

values of both the average number and length of roots were higher than those recorded in autumn.

The root number of autumn cuttings was also affected by the interaction between the hormonal treatment (T) and the shoot portion (P). Indeed, the number of roots per cutting increased from the bottom (B), to the middle (M), and then to the apical (A) portion of collected shoots with the highest value recorded in the apical cuttings treated with T1 (4.5 roots/cutting) followed by T2 treated apical and middle cuttings (3.9 and 3.4 roots/cutting, respectively) (Fig. 6 A), whereas the lowest number of roots was observed in the basal cuttings of untreated control (T0) and T1 (1.5 roots/cuttings) (Fig. 6 A). With respect to the control and T1, a significant increase of the root number was observed in the basal cutting treated with T2 (2.8 roots/cuttings). The capability of olive cuttings on forming adventitious roots is known to be related to the portion (basal, middle or apical) of the shoots, even though different cultivars can respond differentially. A general hypothesis has been formulated on the higher aptitude for rooting of sub-apical cuttings that seems to be related to a possible higher content in auxinic compounds driven basipetally from the young leaves of cuttings (Fabbri *et al.*, 2004). On the other hand, only the root length of spring cuttings was significantly affected by the

interaction between the hormonal treatment (T) and the shoot portion (P) (Table 2, Fig. 6 B), with the highest values (11.4 cm) recorded in the apical cuttings treated with NAA in liquid formulation (T1) and in basal, middle and apical cuttings (10.3, 10.9, and 12.2 cm, respectively) treated with NAD in powdery formulation (T2).

#### 4. Conclusions

This study highlighted that the olive cultivars belonging to autochthonous germplasm of the Campania Region are characterized by a wide variability in the potential rhizogenic activity. Overall the two sampling periods of cutting collection (March and September) significantly affected the rooting aptitude of several cultivars, indicating that in some cultivars the cuttings collected in autumn may have a higher rooting rate than the spring collected ones. Moreover, it was possible to evaluate the rhizogenic aptitude of the cultivars belonging to the olive Campanian germplasm under the influence of rhizogenic treatments. The results showed that the effects of NAA and NAD on rooting strongly depended on interaction with the cultivar, time of collection (autumn or spring) and type of cuttings (basal, medium or apical). In general, the apical and the median portions of shoots were confirmed to be the most suitable for improving the rooting of cuttings.

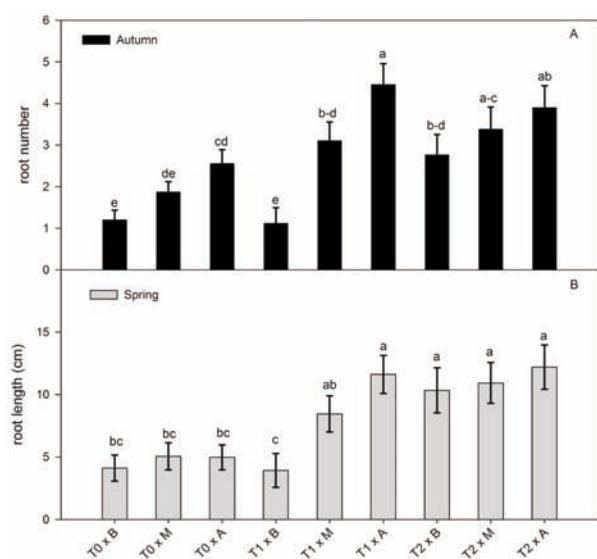


Fig. 6 - Effects of the interaction of the hormonal rhizogenic treatment (T0= Control - no hormonal treatments; T1= NAA treatment; T2= NAD treatment) and shoot portion used to make the cuttings (B= Basal; M= Medium; A= Apical) on root number/cutting (A) of semi-hardwood cuttings collected in autumn and on root length/cutting (B) of semi-hardwood cuttings collected in spring. Means followed by different letters are significantly different for  $P \leq 0.05$  (root number) and  $P \leq 0.05$  (root length).

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