# Effect of Paclobutrazol on Adventitious Root Formation of IBA-Treated Cuttings of 'Zard' and 'Dakal' Olive (*Olea europaea* L.) Cultivars

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**ABSTRACT –** ***Olive cuttings are difficult to propagate. They root well using synthetic auxin, indole-3-butyric acid (IBA). To evaluate the rooting and viability of olive semi-hardwood cuttings of 'Dakal' and 'Zard' cultivars in response to application of growth retardant, paclobutrazol (PBZ), and interaction of IBA and PBZ, a greenhouse experiment was conducted in 2012. Cuttings were dipped for 30 minutes in PBZ (0, 1000, 2000, and 4000 mg.L$^{-1}$) and for 50 seconds in IBA (2000 mg.L$^{-1}$) solutions, respectively and then planted in a sandy bed of propagation under automatic mist system with 95% humidity and 20±5 $^{o}$C base temperature. Results showed that cutting viability, percentages of rooted, callused and rooted plus callused cuttings and root growth parameters were higher in 'Dakal' cultivar than 'Zard' cultivar. The 2000 mg.L$^{-1}$ PBZ treated cuttings had a higher cutting viability, rooting percentage, number of branches per cutting, shoot length, number of roots per cutting, root length and root fresh and dry weights than the control. It appears that the rooting of 'Dakal' cultivar can be substantially improved by the treatment of PBZ (included IBA).***

**Keywords –** olive, auxin, paclobutrazol, semi-hardwood cuttings

## 1. INTODUCTION

Olive (*Olea europaea* **L.**) is one of the most important fruit crops of the Mediterranean basin and parts of Asia Minor (e.g. Iran, Syria, Turkey and Iraq) (Piotto and Noi, 2001), cultivated for table consumption, oil extraction and as ornamental tree (Westwood, 1993). Current olive groves are estimated at approximately 960 million olive trees, of which some 945 million (98% of the total), are found in the Mediterranean basin countries where they cover approximately 9.3 million hectare (Barranco et al., 2010). There are different olive cultivars in Iran, but the 'Zard' and 'Dakal' are dominant cultivars in Shiraz province. The major problem in their cultivation is propagation through cutting because of low rooting capacity. None of the cultivated varieties can be propagated by seed (Sadeghi, 2002) because they revert to the juvenile stage and small-fruited wild type (Sebastiani et al., 2002). Mist propagation of cuttings is the commonly practiced method to propagate many olive cultivars (Gerakakis and Ozkaya, 2005). Using cutting propagation, the nursery production of olive can be sped up and the new plants can bear fruit in four years (Sebastiani et al., 2002).

Adventitious root induction and initiation on stem cuttings of woody species are complex physiological, genetic and environmental processes (Pijut et al., 2011). Many economically important fruit tree species have a low genetic or physiological capacity for adventitious root formation.

Different classes of plant growth regulators have been proven to influence root initiation. To date, auxins have been shown to have the greatest effect on rooting (Basra, 2000).Olive cuttings root well using synthetic auxin indole-3-butyric acid (IBA) (Fabbri et al., 2004), but in difficult-to-root cultivars the auxin either fails to promote rooting or promotes it only slightly (Wiesman and Lavee, 1995).

Hartmann et al. (2011) reported that in IBA-treated cuttings the number of roots was high but their growth was reduced in comparison with untreated cuttings. Application of some plant growth retardants together with IBA has been reported to improve rooting ability and survival in several plant species. Triazoles have been reported to affect some physiological processes during rooting (Wiesman and Lavee, 1994). It has been hypothesized that paclobutrazol (PBZ), a gibberellin synthesis inhibitor (Arteca, 1996) is involved in maintaining low levels of the rooting inhibitor, gibberellins,

in the cuttings (Davis et al., 1988) and in increasing the sink capacity of the base of the cuttings for carbohydrate and/or hormones (Wiesman and Lavee, 1994).

It is well documented that a delicate balance between endogenous stimulatory and inhibitory factors controls the rooting of cuttings (Eliasson, 1981).This study was conducted to study the effects of PBZ and interaction of PBZ and genotype on adventitious root formation in IBA-treated olive cuttings of 'Zard' and 'Dakal' cultivars.

## 2. MATERIALS AND METHODS

### *2.1 Plant Material*

One-year-old olive shoots were collected in November 2012 from mature 'Zard' and 'Dakal' trees, considered as cultivars with low rooting ability. Semi-hardwood cuttings were prepared with 4 to 6 leaves and 15 – 20 cm in length and about pencil-size in diameter. Each cutting was rinsed with common water and after drying sprayed with Diazinon® and Benlate®.

### *2.2 Cutting Treatments*

Solutions of 1000, 2000 and 4000 mg.$L^{-1}$PBZ were freshly made by dissolving Cultar® (active ingredient 250 mg.$L^{-1}$ PBZ) in water. Indole-3-butyric acid solution at 2000 mg.$L^{-1}$ was freshly prepared dissolving IBA powder (Sigma Co., Germany) in ethanol then in water. A few drops of ammonia were added to solution to avoid IBA crystallization.

Semi-hardwood cuttings were immediately dipped into the PBZ different solutions for 30 minutes, wetting 2 cm of their basal end. After receiving PBZ treatments, cuttings were dipped in IBA solution for 50 seconds.

Cuttings were placed 5 cm deep in sand on a bench of a rooting greenhouse equipped with an automatic mist system.

### *2.3 Experimental Design and Statistical Analysis*

This greenhouse experiment was carried out in Isfahan University of Technology, arranged in a 2 x 4 factorial with a completely randomized design with four replications and twenty cuttings per each replicate.

Data were collected three months after planting. Analysis of variance was performed using the General Linear Model procedure of SAS (SAS, Cory, NC, Version 9) and the MSTAT-C software package. Significant differences between means were determined at $P$=0.05 according to the least significant difference (LSD) test.

## 3. RESULTS AND DISCUSSION

The analysis of variance of rooting data showed that cultivar and PBZ significantly affected cutting viability, branching of cutting, number of branches per cutting, average branch length, percentages of rooted cutting, callused cutting and rooted plus callused cutting, number of roots per cutting, mean length of root per cutting, and mean fresh and dry weights of roots per cutting (Table 1). Also, ANOVA of the data revealed that interaction of cultivar and PBZ significantly influenced all studied parameters with exception cutting viability, percentage of callused cuttings and percentage of rooted plus callused cuttings (Table 1).

### *3.1 Cutting Viability*

Mean comparison revealed that cutting viability of 'Dakal' cultivar (86.87%) was higher than 'Zard' cultivar (81.87%) (Table 2). Also, results showed that highest cutting viability (94.37%) obtained on cuttings treated with 2000 mg.$L^{-1}$ PBZ demonstrated 20.52 % increase compared to control (Table 2). The highest cutting viability for both cultivars occurred when cuttings were treated with 2000 mg.$L^{-1}$ PBZ (Table 2). Hafeez et al. (1991), Mukhtar et al. (1998) and Ayaz et al. (2004) reported cutting viability of guava with PBZ treatment. High root formation means high ability to absorb water and nutrients. That is why the leaves of successful cuttings remained intact and also started fresh growth (Ayaz et al., 2004).

### *3.2 Number of Branches per Cutting*

Mean comparison showed that number of branches per cutting was higher in 'Dakal' than in 'Zard' (Table 2).PBZ at 2000 mg.$L^{-1}$ gave the highest number of branches per cutting followed by PBZ at 4000 mg.$L^{-1}$. In comparison to the control, treatment with PBZ at 2000 mg.$L^{-1}$ concentration, increased number of branches per cutting up to 46.64 % (Table 2). Using 2000 and 4000 mg.$L^{-1}$ PBZ caused higher shoot number per cutting in 'Dakal' while 2000 mg.$L^{-1}$ PBZ, resulted highest shoot number per cutting in 'Zard' (Table 2). Basra (2000) reported that treatment with PBZ was associated with increase in cytokinins levels and cytokinin production resulted in the increase in number of branches. Podwyszynska and Marasek (2003) found that adding paclobutrazol to culture medium of tulip flower explants (containing thidiazuron), significantly enhanced the number of shoot meristems. Ayaz et al. (2004) reported that the number of branches per cutting was positively correlated with increasing root mass which is important for survival and environmental adaptation of plants.

### *3.3 Shoot Length*

Mean length of shoots formed on rooted cuttings was 6.012 cm for 'Dakal' and 2.99cm for 'Zard' cultivar (Table 2). PBZ treatment of 2000 mg.$L^{-1}$ significantly differed from other treatments with maximum shoot length (6.57 cm) against minimum shoot length (2.4 cm) in control (Table 4). The maximum shoot growth in 'Dakal' (7.94 cm) and 'Zard' (5.2 cm) cultivars were recorded when the cuttings were treated with2000 mg.$L^{-1}$ PBZ (Table4).

Although PBZ is known to suppress shoot growth of most plants, the increase in number of shoots in PBZ-treated cuttings can cause an increase in the total shoot length. This result is in agreement with the findings of Baninasab and Ghobadi (2011), Marshall and Waring (1985) and Rahman et al. (2004). Baninasab and Ghobadi (2011) reported an increment in growth of cucumber seedlings with paclobutrazol treatment. Rahman et al. (2004) also reported maximum number of branches per cutting of guava cuttings with the application of paclobutrazol. More number of leaves and roots may have resulted in high photosynthesis and more photosynthate material. The increasing photosynthate material may have shown its impact on length of shoots as described by Marshall and Waring (1985).

### *3.4 Percentages of Rooted and Callused Cuttings*

When data were analyzed separately for selected cultivars, 23.43% rooting was observed among cuttings of 'Dakal' (Table 2). Rooting percentage was higher among cuttings of 'Dakal' in comparison to 'Zard'(Table 2). Also, maximum rooting percentage (26.25%) was recorded in cuttings treated with 2000 mg.$L^{-1}$ PBZ, in comparison to minimum (8.87 %) in control demonstrated more than three times increase (Table 2). Mean comparison showed that more callused cuttings (17 %) were produced in 'Dakal' cultivar than in 'Zard' (14 %) (Table 2). The maximum percentage of callused cuttings was obtained with 2000 mg.$L^{-1}$ PBZ (Table 2). The application of some growth retardants (include PBZ) together with IBA has been used to improve the rooting capacity of cuttings in some species (Henrique et al., 2006). It has been suggested that the cause of the synergistic effect of IBA plus PBZ treatment might be due to increased endogenous auxin level (Pan and Tian, 1999). Pan and Tian (1999) reported that IBA together with PBZ treated cuttings rooted at a higher frequency than those treated with IBA. Botelho et al. (2005) also reported rooting increment in vine rootstock '43-43' cuttings with IBA plus PBZ treatment. Wiesman and Riov (1994) suggested that PBZ may affect the rate of metabolism of IBA during rooting and the status of the local sink in the base of the cuttings, thus partially contributing to the enhancement of the rooting-promotive effect of IBA.

### *3.5 Number of Roots per Cutting*

The mean number of roots per cutting was significantly higher in 'Dakal' than in 'Zard' (Table 3). More roots developed on cuttings of 'Dakal' (10.2) and 'Zard' (9.8) when treated with 2000 mg.$L^{-1}$ PBZ than the other concentrations (Table 3). According to Bora et al. (1991), enhancement of adventitious root number by PBZ is accompanied by changes in enzyme activities (such as phosphatase, polyphenol oxidase) and level of chemical constituents (e.g. soluble sugars and phenolic compound) which have been involved in rooting. Wiesman et al. (1995) reported that paclobutrazol application increased number of roots per cutting in avocado. The increase in number of roots is necessary to provide anchor to the high shoot length and to keep shoot-root ratio in balance (Ayaz et al, 2004).

### *3.6 Root Length*

Cuttings of 'Dakal' cultivar produced the longest roots (3.69 cm) (Table 3). Roots of 'Dakal' cuttings exposed to 4000 mg.$L^{-1}$ PBZ appeared to be longer compared to cuttings exposed to 0, 1000 or 2000 mg.$L^{-1}$ PBZ. However, roots of 'Zard' cuttings exposed to 2000 mg.$L^{-1}$ PBZ had the greatest length of all treatments (Table 3).These results showed that the total root length was increased in cuttings due to increment in root number per cutting but in case of root length, paclobutrazol has been shown to reduce root length. Depending on the plant species and the concentration, PBZ either stimulated or inhibited root growth (Basra, 2000). Arteca (1996) and Davis (2004) reported that auxin more influenced cell elongation which causes increase in root growth. Abdi and Ascari-Raburi (2009) showed that treating poinciana (*Delonix regia*) stem cuttings with paclobutrazol plus indole-3-butyric acid produced higher total root length through increasing number of newly formed roots than in cuttings, treated with IBA alone. Geneva (1990) also observed that PBZ caused increasing of English ivy root growth.

### *3.7 Fresh Weight of Root*

Cuttings of 'Dakal' exhibited higher root fresh weight than 'Zard' (Table 3). Application of PBZ, regardless of the rate (1000, 2000 or 4000 mg.$L^{-1}$) improved fresh weight of roots of both cultivars studied (Table 3). $GA_3$ inhibited rooting, thus gibberellins inhibitors have been reported to increase the root growth in various plants (Hartmann et al. 2011). Martinez et al. (2003) reported that application of paclobutrazol increased root density and root width in cuttings of *Rhamnus alaternus*.

### *3.8 Dry Weight of Root*

There was significant difference between 'Dakal' and 'Zard' cultivars in terms of root dry weight. PBZ at 2000 mg.$L^{-1}$ gave the highest dry weight of roots (Table 3). Highest root dry weight for 'Dakal' obtained when cuttings were treated with 1000 mg.$L^{-1}$PBZ while in the case of Zard maximum dry weight of roots was obtained with 2000 mg.$L^{-1}$ PBZ (Table 3). Our results are in agreement with Tagliavini and Looney (1991), Fuller and Zajicek (1995) and Martinez et al. (2003) who showed that PBZ treatment, increased the carbohydrates content of cuttings. Baninasab and Ghobadi (2011) reported an increment in growth of cucumber seedlings with paclobutrazol treatment.

Also analysis of data showed that there was a positive and highly significant correlation among all studied parameters (Table 4).

## 4. CONCLUSION

As mentioned above, application of paclobutrazol, in terms of rooting enhancement, obviously influenced all studied rooting parameters. Application of paclobutrazol at the rate of 2000 mg.$L^{-1}$ substantially increased cutting viability, branches per cutting, mean shoot length, rooting percentage, root number per cutting, mean root length and root fresh and dry weights in comparison with control. It's appeared that paclobutrazol inhibits gibberellin biosynthesis which prevents adventitious root formation and also paclobutrazol application increased the content of endongenous cytokinin which in turn causes cell division and makes roots as sinks that ultimately more diversion of photosynthates occurred to the roots than aerial portions.

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Table 1. Analysis of variance (ANOVA) for cultivar (Cult.), paclobutrazol concentration (PBZ) and their interaction (Cult × PBZ) for rooting parameters

| Source of Variance | DF | Means Squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Cutting Viability | No. of Branches per Cutting | Shoot Length | Rooted Cuttings | Callused Cuttings | No. of Roots per Cutting | Root length | Root Fresh Weight | Root Dry Weight |
| Cult. | 1 | 200.000 * | 0.281 * | 72.782 ** | 1378.12 ** | 94.531 * | 35.806 * | 3.791 * | 0.014 ** | 0.0002 * |
| PBZ | 3 | 543.750 ** | 1.871 ** | 23.270 ** | 480.083 ** | 240.364 ** | 58.348 ** | 1.716 * | 0.060 ** | 0.0012 ** |
| BPZ × Cult | 3 | 14.583 ns | 0.441 * | 4.426 ** | 224.875 ** | 2.864 ns | 14.520 * | 1.873 * | 0.001 ** | 0.0025 ** |
| Error | 24 | 27.604 | 0.058 | 0.255 | 9.312 | 11.718 | 2.082 | 0.439 | 0.00004 | 0.00002 |

*, **, and ns show significantly difference at 5% and 1% levels of probability and non-significance, respectively.

Table 2. Effects of cultivar, PBZ and their interaction on different rooting parameters

| Cultivar | PBZ (mg/L) | | | | Mean |
|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 4000 | |
| | | | Cutting Viability (%) | | |
| Zard | 72.500 a* | 80.000 a | 92.500a | 82.500a | 81.875 B |
| Dakal | 77.500 a | 82.500a | 96.250 a | 91.250a | 86.875 A |
| Mean | 75.000 D | 81.250 C | 94.375 A | 86.875 B | |
| | | | No. of Branches per Cutting | | |
| Zard | 1.250 e | 1.600 c | 2.500 a | 1.500 d | 1.712 B |
| Dakal | 1.325 e | 1.575 cd | 2.325 b | 2.375 b | 1.900 A |
| Mean | 1.287 D | 1.587 C | 2.412 A | 1.937 B | |
| | | | Shoot Length (cm) | | |
| Zard | 1.85 g | 2.47 f | 5.2 d | 2.45 f | 2.996 B |
| Dakal | 3.09 e | 7.32 b | 7.94 a | 5.68 c | 6.012 A |
| Mean | 2.4 D | 4.9 B | 6.571 A | 4.06 C | |
| | | | Rooted Cuttings (%) | | |
| Zard | 6.25 g | 12.50 d | 12.50 d | 10 f | 10.31 B |
| Dakal | 11.50 e | 27.25 b | 40 a | 15 c | 23.43 A |
| Mean | 8.87 D | 19.87 B | 26.25 A | 12.50 C | |
| | | | Callused Cuttings (%) | | |
| Zard | 7.500 a | 15.000 a | 20.000 a | 13.750 a | 14.063 B |
| Dakal | 10.000 a | 20.000 a | 23.750 a | 16.250 a | 17.500 A |
| Mean | 8.750 C | 17.500 B | 21.875 A | 15.000 B | 14.063 B |

* Means that have at least one similar uppercase or lowercase letter within column or row, are non-significant at 5% level of probability according to LSD test.

Table 3. Effects of cultivar, PBZ and their interaction on different rooting parameters

| Cultivar | PBZ (mg/L) | | | | Mean |
|---|---|---|---|---|---|
| | 0 | 1000 | 2000 | 4000 | |
| | | | No. of Roots per Cutting | | |
| Zard | 3.58 e | 2.35 f | 9.8 a | 4.41 d | 5.03 B |
| Dakal | 3.98 de | 8.41 b | 10.2 a | 6.1 c | 7.15 A |
| Mean | 3.87 C | 5.38 B | 10 A | 5.21 BC | |
| | | | Root Length (cm) | | |
| Zard | 2.31 f | 2.84 d | 4.3 a | 2.59 e | 3.011 B |
| Dakal | 3.68 b | 3.27 c | 3.72 b | 4.09 a | 3.693 A |
| Mean | 3 B | 3.05 B | 4.01 A | 3.34 AB | |
| | | | Root Fresh Weight (g) | | |
| Zard | 0.187 f | 0.239 d | 0.297 b | 0.210 e | 0.233 B |
| Dakal | 0.149 g | 0.252 c | 0.452 a | 0.250 c | 0.276 A |
| Mean | 0.168 D | 0.246 B | 0.375 A | 0.230 C | |
| | | | Root Dry Weight (g) | | |
| Zard | 0.0132 f | 0.0280 e | 0.0757 a | 0.0137 f | 0.0326 B |
| Dakal | 0.0422 c | 0.0480 b | 0.0285 e | 0.0352 d | 0.0385 A |
| Mean | 0.0277 C | 0.0380 B | 0.0521 A | 0.0245 C | |

* Means that have at least one similar uppercase or lowercase letter within column or row, are non-significant at 5% level of probability according to LSD test.

Table 4. Correlation among studied parameters†

| Parameter †† | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.00000 | | | | | | | | | | | | |
| 2 | 0.97969** | 1.00000 | | | | | | | | | | | |
| 3 | 0.93151** | 0.96454** | 1.00000 | | | | | | | | | | |
| 4 | 0.97999** | 0.97223** | 0.96240** | 1.00000 | | | | | | | | | |
| 5 | 0.97715** | 0.95081** | 0.90308** | 0.97033** | 1.00000 | | | | | | | | |
| 6 | 0.97509** | 0.96552** | 0.92129** | 0.96741** | 0.98580** | 1.00000 | | | | | | | |
| 7 | 0.89864** | 0.89172** | 0.91023** | 0.91117** | 0.83658** | 0.85550** | 1.00000 | | | | | | |
| 8 | 0.89891** | 0.86814** | 0.87767** | 0.91805** | 0.84460** | 0.84963** | 0.97610** | 1.00000 | | | | | |
| 9 | 0.93509** | 0.91965** | 0.88655** | 0.93184** | 0.88431** | 0.89675** | 0.95772** | 0.96248** | 1.00000 | | | | |
| 10 | 0.93284** | 0.96151** | 0.94120** | 0.93537** | 0.88282** | 0.91301** | 0.94509** | 0.91063** | 0.96067** | 1.00000 | | | |
| 11 | 0.91208** | 0.93774** | 0.96057** | 0.93906** | 0.85783** | 0.88699** | 0.96980** | 0.93812** | 0.95061** | 0.98004** | 1.00000 | | |
| 12 | 0.91477** | 0.91574** | 0.93040** | 0.96208** | 0.90200** | 0.89805** | 0.91850** | 0.93975** | 0.94295** | 0.92626** | 0.93960** | 1.00000 | |
| 13 | 0.83428** | 0.79759** | 0.76850** | 0.84557** | 0.84608** | 0.86285** | 0.83040** | 0.86170** | 0.90612** | 0.83646** | 0.82657** | 0.88271** | 1.00000 |

† ** shows highly significantly difference between two parameter.

†† 1=cutting viability; 2=% of branched cuttings; 3= no. shoot per cutting; 4=rooting percentage; 5=% of callused cuttings; 6= rooted plus callused cuttings; 7=shoot fresh weight; 8=root fresh weight; 9=shoot dry weight; 10=root dry weight; 11= mean root length; 12= mean root number per cutting and 13=mean shoot length.