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Effect of Plant Growth Promoting Rhizobacteria and IBA on rooting of cuttings in kiwifruit (*Actinidia deliciosa* Chev.)

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ABSTRACT

The present study was conducted under a polyhouse with kiwifruit cuttings. The entire programme of the study was divided into two experiments comprising hardwood and semi-hardwood cuttings. The experiments were laid out in Randomized Block Design, with three replications per treatment. Experiment I was carried out on hardwood cuttings of kiwifruit cultivar Allison and comprised of nine treatments, viz., T_1 (IBA 5000ppm), T_2 (*Bacillus subtilis*), T_3 (*Bacillus licheniformis*), T_4 (*Bacillus subtilis* + IBA 4000ppm), T_5 (*Bacillus licheniformis* + IBA 4000ppm), T_6 (*Bacillus subtilis* + IBA 3000ppm), T_7 (*Bacillus licheniformis* + IBA 3000ppm), T_8 (*Bacillus subtilis* + IBA 2000ppm) and T_9 (*Bacillus licheniformis* + IBA 2000ppm). In Experiment II, all the above-mentioned nine treatments were imposed on semi-hardwood cuttings of kiwifruit. Ttreatment IBA 5000ppm recorded best root characteristics (per cent rooted cuttings, number of primary roots secondary roots, length of roots total root length, root biomass); shoot characteristics (shoot length, shoot diameter, shoot biomass) and leaf characteristics (number of leaves and leaf area) in both hardwood and semi-hardwood cuttings. This treatment also resulted in maximum net benefit per 100 cuttings in comparison to other treatments. Among the two types of cuttings studied, hardwood cuttings showed better results on root characteristics. However, semi-hardwood cuttings gave better results on shoot and leaf characteristics.

Key words: PGPR, kiwifruit, *Actinidia deliciosa*, IBA, cuttings

INTRODUCTION

Kiwifruit (*Actinidia deliciosa* Chev.) is a dioecious and deciduous vine, native to China (Ferguson, 1984). The kiwifruit has gained enormous popularity in the recent past due to its wide climatic-adaptability, unique blend of taste, and high medicinal value. Availability of quality planting material is the first and foremost priority for commercializing any fruit crop. An increasing demand for kiwifruit plants has necessitated development of easier, quicker and economic methods of propagation. Although there are various methods of propagation for multiplying fruit crops such as grafting, budding or tissue culture, most are expensive, time-consuming, laborious, and require skill. The most rapid and suitable method of multiplication of fruit crops is through cuttings, especially hardwood and semi-hardwood cuttings. In general, semi-hardwood cuttings in kiwifruit are known to yield higher rooting-success than hardwood cuttings. Higher rooting potential of semi-hardwood cuttings is attributed to production of endogenous auxins (Hartmann *et al*, 1983).

Use of exogenous plant growth regulators for enhancing success rate in cuttings is a very commonly used method in fruit crops (Polat and Kamiloglu, 2007). Indole-3-butyric acid (IBA) is the best auxin for this purpose, as, it is non-toxic to plants over a wide range of concentrations, and effectively in promotes rooting in a large number of plant species (Hartmann *et al*, 2002). Plant Growth Promoting Rhizobacteria (PGPR) is another option for enhancing per cent success in rooted cuttings. PGPR are naturally-occurring soil bacteria that aggressively colonize plant roots, and benefit plants by providing them growth promotion (Cleyet-Marel *et al*, 2001). Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through their direct effect on root and shoot growth. One of the most characteristic effects of PGPR is increased elongation rate, and consequently, enhanced initiation rate of lateral roots, resulting in a more branched root-architecture (Kapulnik *et al*, 1985; Lifshitz *et al*, 1987). With this in view, the present investigation was undertaken to evaluate the effect of Plant Growth Promoting

Rhizobacteria, alone and in combination with IBA, on rooting and growth in kiwifruit cuttings.

MATERIAL AND METHODS

The experiments were conducted in the Kiwifruit Block of Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), during 2011-2012. The entire study was carried out in two experiments comprising hardwood and semi-hardwood cuttings. Cuttings, with uniform vigour and size were taken from 27-year-old vines of 'Allison' cultivar of kiwifruit. Experiments were laid out in Randomized Block Design, with three replications per treatment. Experiment I was carried out on hardwood cuttings and comprised nine treatments, viz., T_1 (IBA 5000ppm), T_2 (*Bacillus subtilis*), T_3 (*Bacillus licheniformis*), T_4 (*Bacillus subtilis* + IBA 4000ppm), T_5 (*Bacillus licheniformis* + IBA 4000ppm), T_6 (*Bacillus subtilis* + IBA 3000ppm), T_7 (*Bacillus licheniformis* + IBA 3000ppm), T_8 (*Bacillus subtilis* + IBA 2000ppm), and T_9 (*Bacillus licheniformis* + IBA 2000ppm). Experiment II, all the above-mentioned nine treatments were imposed on the semi-hardwood cuttings.

Plant Growth Promoting Rhizobateria - PGPR - (500ml each of *Bacillus subtilis* and *Bacillus licheniformis*) were used for treating kiwifruit cuttings. Bacterial cell suspension (O.D. value 2.0 at 540nm) of 48-hour old culture grown on nutrient agar medium plates @10% was used as the inoculums, which contained viable cells upto a dilution of 10^8cfu/ml. These cuttings were dipped in PGPR solution for 4 hours, and, were thereafter planted in the experimental field. For combined treatment of IBA and PGPR, the cuttings were first treated with IBA solution as a quick dip, and then again dipped in PGPR solution for upto 2cm length, for 4 hours. The cuttings were then removed from the solution and planted under a polyhouse.

In both the experiments, mature dormant shoots 25-30cm long, 0.5-1.0cm thick having at least three healthy, bold buds were selected during mid-January and mid July, respectively. The cuttings were taken from plants under shade. The base of the cutting was given a round cut near the bud, whereas, a slanting cut was made at the top of the cutting to prevent water accumulation and rotting. Two to three leaves were retained in semi-hardwood cuttings to reduce transpiration loss and overlapping of leaves during planting of the cuttings in beds. Leaf area was reduced by cutting leaves to half their size. The cuttings, after dipping in different solutions as per experimental details, were planted in a bed at 2m x 1m, containing sand, forest soil and FYM in 1:1:1 ratio, under polyhouse conditions with shade, ventilation and irrigation arrangement. Rooted cuttings obtained from both the experiments were uprooted in the last week of December. Data from the present investigation were subjected to statistical analysis as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Root characteristics

Results revealed that IBA alone or in combination with plant growth promoting Rhizobacteria exerted significant influence on various root characteristics of cuttings in kiwifruit (Table 1). Highest percentage of rooted cuttings, i.e., 46.3% and 62.4%, in the case of hardwood and semi-hardwood cuttings, respectively, was recorded with IBA 5000ppm (T_1). This treatment also resulted in the highest number of primary roots (8.5 and 12.6), number of secondary roots (18.3 and 23.7), longest root (20.5cm and 24.6cm), total root length (135.7cm and 141.4cm), fresh weight of roots (12.0g and 13.3g), and dry weight of roots (6.0g and 11.3g) in hardwood and semi-hardwood cuttings, respectively, followed by the treatment *Bacillus subtilis* + IBA 4000ppm (T_4).

Hartmann *et al* (2002) opined that as the annual growth-cycle progresses, endogenous auxin production begins to decrease and, therefore, high levels of exogenous auxins are needed to promote rooting. Application of auxin is the most common treatment for enhancing rooting in stem cuttings. In addition to this, auxin affects cell differentiation,

Table 1. Effect of Plant Growth Promoting Rhizobacteria and IBA on rooting of cuttings in kiwifruit

Treatment code	Treatment	Per cent rooted cuttings	
		Hardwood	Semi-hardwood
T_1	IBA 5000ppm	46.3 (42.9)*	62.4 (52.2)
T_2	*Bacillus subtilis*	7.7 (16.1)	11.5 (19.8)
T_3	*Bacillus licheniformis*	5.5 (13.6)	8.5 (16.9)
T_4	*Bacillus subtilis* + IBA 4000ppm	44.5 (41.8)	59.7 (50.6)
T_5	*Bacillus licheniformis* + IBA 4000ppm	38.3 (38.2)	50.5 (42.3)
T_6	*Bacillus subtilis* + IBA 3000ppm	42.5 (40.6)	54.4 (47.5)
T_7	*Bacillus licheniformis* + IBA 3000ppm	30.5 (33.5)	42.2 (40.5)
T_8	*Bacillus subtilis* + IBA 2000ppm	35.2 (36.4)	47.3 (43.5)
T_9	*Bacillus licheniformis* + IBA 2000ppm	26.7 (31.1)	38.5 (38.4)
CD (P=0.05)		2.0	1.7
SEm (+)		0.67	0.58
CV (%)		3.75	2.40

*Figures in parentheses are arc sine transformed values

and promotes starch hydrolysis and mobilization of sugars / nutrients to the base of the cutting (Das *et al*, 1997). Highest per cent rooted cuttings and number of primary and secondary roots obtained with IBA 5000ppm treatment in the present study are supported by Rana *et al* (1999) who reported dormant cuttings treated with IBA 5000ppm to express better rooting and survival percentage in five kiwifruit cultivars, namely, Monty, Bruno, Hayward, Abbott and Allison.

Data presented in Table 2 reveals that the highest (8.5 and 12.6) number of primary roots were recorded with the treatment IBA 5000ppm (T_1), and this was statistically at par with the treatment *Bacillus subtilis* + IBA 4000ppm (T_4), exhibiting 7.9 and 10.8 number of primary roots; the lowest (3.5 and 4.4) number of primary roots were recorded with the treatment *Bacillus licheniformis* (T_3), followed by *Bacillus subtilis* (T_2), recording on average 4.1 and 4.7 primary roots in hardwood and semi-hardwood cuttings, respectively. Highest number of secondary roots both in hardwood and semi-hardwood cuttings was also recorded with IBA 5000ppm (T_1), which was statistically at par with *Bacillus subtilis* + IBA 4000ppm (T_4).

In hardwood cuttings, length of the longest root per cutting ranged between 4.1cm and 20.5cm, whereas, it varied between 6.5cm and 24.6cm in semi-hardwood cuttings. The longest (20.5cm) root was recorded in the treatment IBA 5000ppm (T_1) in hardwood cuttings, which was followed by the treatment *Bacillus subtilis* + IBA 4000ppm (T_4). In the case of semi-hardwood cuttings also, the longest (24.6cm) root was recorded with the treatment IBA 5000ppm (T_1) (Table 2). In the present study, the lowest rooting performance obtained with application of solely *Bacillus subtilis* or *Bacillus licheniformis* may be because strains of the bacteria used under the study were not able to colonize the cuttings of kiwifruit. Kapulnik *et al* (1985) and Lifshitz *et al* (1987) also reported that Plant Growth Promoting Rhizobacteria could not initiate rooting, but could only colonize developed roots, and promote their growth. The mechanism of root induction by PGPR is, however, not completely understood; but, it is thought to be due to production of phytohormones, inhibition of ethylene synthesis, and mineralization of nutrients by PGPR (Goto, 1990; Steenhoudt and Vanderlayden, 2000).

In hardwood and semi-hardwood cuttings, the highest (135.7cm and 141.4cm) value for total root length was recorded with the treatment IBA 5000ppm (T_1), followed by the treatment *Bacillus subtilis* + IBA 4000ppm (T_4) which recorded 127.2cm and 132.4cm total root length per cutting, respectively. It is evident from Figures 1 & 2 that the highest fresh and dry weight of roots per cutting was recorded with the treatment IBA 5000ppm (T_1) in both the hardwood and semi-hardwood cuttings. These results are in line with Siddiqui and Hussain (2007) who reported maximum root length in cuttings of *Ficus hawaii* with IBA 4000ppm. They also speculated that the higher root length was due to an effect of IBA on carbohydrate metabolism and translocation.

Table 2. Effect of Plant Growth Promoting Rhizobacteria and IBA on root characteristics of cuttings in kiwifruit

Treatment code	Treatment	PrimaryRoots		Secondary Roots		Longest root (cm)		Total root length (cm)	
		Hardwood	Semi-hardwood	Hardwood	Semi-hardwood	Hardwood	Semi-hardwood	Hardwood	Semi-hardwood
T_1	IBA 5000ppm	8.5	12.6	18.3	23.7	20.5	24.6	135.7	141.4
T_2	*Bacillus subtilis*	4.1	4.7	6.5	11.2	6.5	10.7	37.4	43.6
T_3	*Bacillus licheniformis*	3.5	4.4	5.3	8.5	4.1	6.5	29.6	34.1
T_4	*Bacillus subtilis* + IBA 4000ppm	7.9	10.8	16.7	21.8	17.9	22.0	127.2	132.4
T_5	*Bacillus licheniformis* + IBA 4000ppm	6.8	9.6	12.1	15.7	12.5	16.5	105.7	109.5
T_6	*Bacillus subtilis* + IBA 3000ppm	7.3	8.8	13.3	17.4	15.2	18.7	120.9	123.8
T_7	*Bacillus licheniformis* + IBA 3000ppm	6.2	6.9	8.3	13.7	9.5	14.5	86.3	92.6
T_8	*Bacillus subtilis* + IBA 2000ppm	6.6	7.2	10.0	14.4	11.5	15.9	94.6	96.3
T_9	*Bacillus licheniformis* + IBA 2000ppm	5.9	6.8	7.3	12.0	8.0	11.6	77.5	80.7
CD (P=0.05)		2.0	2.1	2.3	2.1	1.7	2.8	1.2	1.5
SEm (+)		0.68	0.69	0.77	0.71	0.58	0.93	0.39	0.51
CV (%)		18.55	16.24	12.16	7.98	8.65	10.58	0.74	0.93

Shoot and leaf characteristics

Highest (23.8cm and 20.5cm) shoot length in both hardwood and semi hardwood cuttings was recorded with the treatment IBA 5000ppm (T_1), which was statistically at par with *Bacillus subtilis* + IBA 4000ppm (T_4), resulting in shoot length of 22.5cm and 19.0cm, respectively. Data presented in Table 3 shows that the highest (10.6mm and 8.5mm) shoot diameter in hardwood and semi-hardwood cuttings was recorded with the treatment IBA 5000ppm (T_1), which was statistically at par with *Bacillus subtilis* + IBA 4000ppm (T_4), respectively. Better shoot characteristics seen in cuttings treated with different concentrations of IBA alone or in combination with PGPR may be attributed to better rooting performance perhaps consequent to higher carbohydrate production and assimilation.

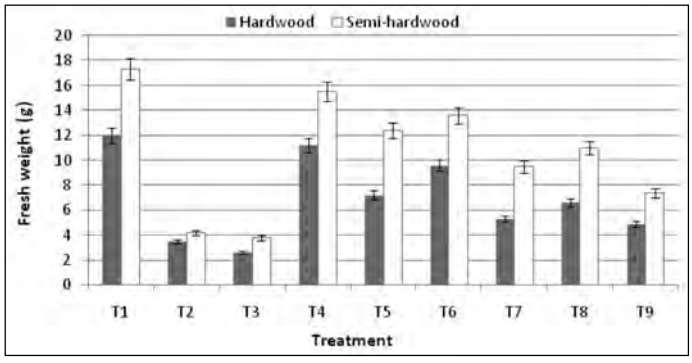

Fig. 1. Effect of Plant Growth Promoting Rhizobacteria and IBA on fresh weight of roots per cutting in kiwifruit

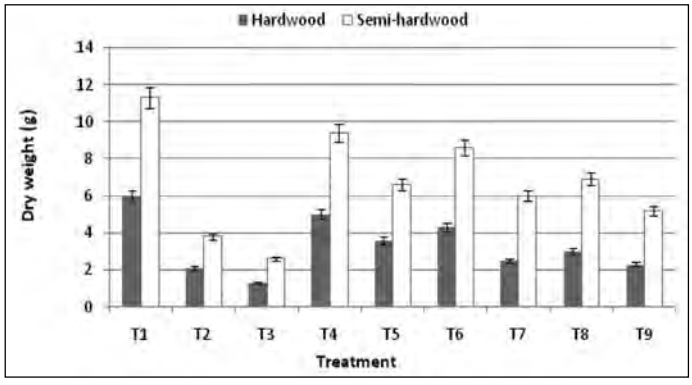

Fig. 2. Effect of Plant Growth Promoting Rhizobacteria and IBA on the dry weight of roots per cutting in kiwifruit

Fresh weight of shoot ranged from 8.4g to 25.8g in hardwood cuttings, and from 6.5g to 21.3g in semi-hardwood cuttings in kiwifruit cv. 'Allison'. Dry weight of shoot ranged from 3.4g to 15.3g in hardwood cuttings, and from 2.6g to 10.7g in semi-hardwood cuttings. Perusal of data presented in Figures 3 and 4 reveals that the highest fresh and dry weight of shoots per cutting was recorded in the treatment IBA 5000ppm (T_1) in both types of cuttings, viz., hardwood and semi-hardwood. It is also evident from Table 3 that the highest (15.6 and 12.4) leaf number in both hardwood and semi-hardwood cuttings was recorded with IBA 5000ppm (T_1), respectively. The highest (168.9cm^2 and 166.7cm^2) leaf area in both types of cutting was attained with the treatment IBA 5000ppm (T_1), followed by the treatment *Bacillus subtilis* + IBA 4000ppm (T_4) exhibiting leaf area of 155.6cm^2 and 150.0cm^2, respectively.

These results are in conformity with investigations of Alam *et al* (2007) who obtained maximum number of leaves

Table 3. Effect of Plant Growth Promoting Rhizobacteria and IBA on shoot and leaf characteristics in kiwifruit cuttings

Treatment code	Treatment	Shoot length (cm)		Shoot diameter (mm)		Average leaf area (cm^2)		Leaf number	
		Hardwood	Semi-hardwood	Hardwood	Semi-hardwood	Hardwood	Semi-hardwood	Hardwood	Semi-hardwood
T_1	IBA 5000ppm	23.8	20.5	10.6	8.5	168.9	166.7	15.6	12.4
T_2	*Bacillus subtilis*	8.6	6.5	4.6	3.9	19.8	17.6	7.0	4.9
T_3	*Bacillus licheniformis*	6.0	4.9	3.7	2.6	16.3	15.6	6.0	3.9
T_4	*Bacillus subtilis* + IBA 4000ppm	22.5	19.0	8.8	7.2	155.6	150.0	13.3	10.6
T_5	*Bacillus licheniformis* + IBA 4000ppm	16.5	12.1	8.2	4.6	113.4	110.7	9.8	6.1
T_6	*Bacillus subtilis* + IBA 3000ppm	20.4	16.7	8.5	5.5	145.6	143.0	11.5	7.2
T_7	*Bacillus licheniformis* + IBA 3000ppm	16.7	12.8	7.7	4.4	96.4	90.2	11.0	6.7
T_8	*Bacillus subtilis* + IBA 2000ppm	12.5	10.5	7.5	4.3	89.5	87.0	9.8	5.8
T_9	*Bacillus licheniformis*+ IBA 2000ppm	12.0	9.5	7.0	4.0	77.5	73.3	9.4	5.0
CD (P=0.05)	1.7	2.1	2.1	1.2	1.5	1.8	2.4	1.8	
SEm (+)		0.58	0.71	0.69	0.38	1.54	0.60	0.80	0.59
CV (%)		6.49	9.79	16.24	13.31	0.89	1.09	13.44	14.57

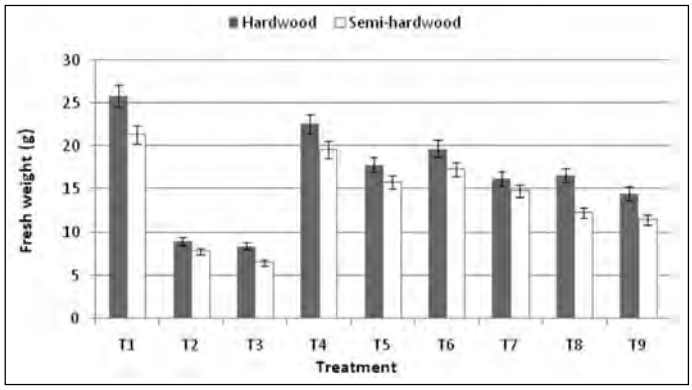

Fig. 3. Effect of Plant Growth Promoting Rhizobacteria and IBA on fresh weight of shoots per cutting in kiwifruit

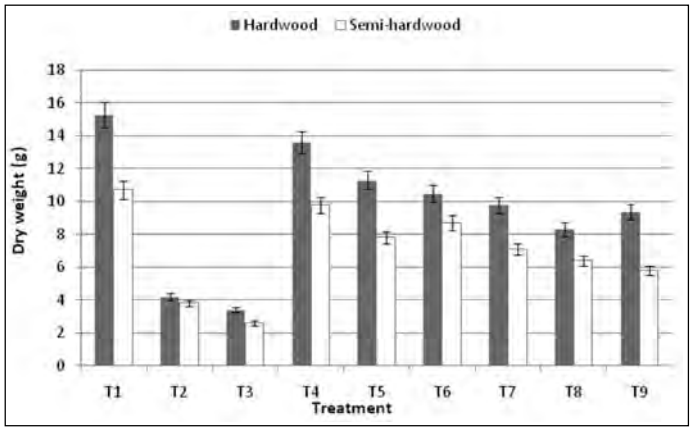

Fig. 4. Effect of Plant Growth Promoting Rhizobacteria and IBA on dry weight of shoots per cutting in kiwifruit

in kiwi cuttings treated with IBA 4000ppm. They attributed their results to development of a better root system which may have played an active role in development of leaves by facilitating supply of water and nutrients to the cuttings from the soil. Similar trend was observed with application of auxins in semi-hardwood cuttings of guava, with enhanced leaf number (Taiz and Zeiger, 2006).

Effect of season on hardwood and semi-hardwood cuttings

Results in the present study indicated that the season in which cuttings were made also had a significant influence on root, shoot and leaf characteristics in kiwifruit. The best rooting performance in terms of per cent rooted cuttings, number of primary and secondary roots, length of the longest root, total root length, fresh and dry weight of roots and shoots per cutting were recorded in semi-hardwood cuttings prepared in mid-July, whereas, the best results for various shoot and leaf characteristics, viz., shoot length, shoot diameter, shoot biomass, leaf number and leaf area, were noticed with hardwood cuttings prepared in mid-January.

The type of wood, stage of growth and the time of year when cuttings are taken are some of the important factors for obtaining satisfactory rooting pattern in plants (Hartmann *et al*, 2002). Fouad *et al* (1990) studied seasonal fluctuations in rooting ability in eight olive cultivars. They reported that the date of cuttings had a great influence on their rooting ability. Cuttings prepared in summer, especially in August, gave the highest rooting percentage, while those prepared in January rooted the least. Singh *et al* (2008) also reported that cuttings prepared during the active growth-stage gave better results in respect of rooting percentage and number of roots per cutting in olive.

Results obtained in the present study showed that IBA 5000ppm gave the best results in root, shoot and leaf characteristics in both hardwood and semi-hardwood cuttings. However, results obtained on different root characteristics of cuttings treated with *Bacillus subtilis* + IBA 4000ppm were statistically non-significant with results obtained with IBA 5000ppm. Application of *Bacillus subtilis* or *Bacillus licheniformis* solely recorded the lowest values for various root, shoot or leaf characteristics. Among the two types of cuttings tested, superior root characteristics were recorded in semi-hardwood cuttings.

REFERENCES

Alam, R., Khalil, U.R., Muhammad Ilyas, Muhammad Ibrahim and Muhammad, A.R. 2007. Effect of IBA concentrations on the rooting of kiwi cuttings. *Sarhad J. Agri.*, **23**:293-295

Cleyet-Marel, J.C., Larcher, M., Bertrand, H., Rapior, S. and Ponichet, X. 2001. Plant growth enhancement by Rhizobacteria. In: Nitrogen assimilation by plants: physiological, biochemical and molecular aspects. Science Publishers, En field (NH, USA), Plymouth (UK) pp. 184-197

Das, P., Basak, U.C. and Das, A.B. 1997. Metabolic changes during rooting in pre-girdled stem cuttings and air-layers of *Heritiera*. *Botanical Bulletin of Academia Sinica*, **38**:91-95

Ferguson, A.R. 1984. Kiwifruit: a botanical review. *Hort. Rev.*, **6**:1-64

Fouad, M.M., Fayek, M.A., Selin, H.H. and El-Sayed, M.E. 1990. Rooting of eight olive cultivars under mist. *Acta Hort.*, **286**:57-60

Gomez, K.A. and Gomez, A.A. 1984. In: Statistical Procedure for Agricultural Research, 2nd ed., Wiley Interscience, New York, pp. 304-309

Goto, M. 1990. Fundamentals of bacterial plant pathology. Academic Press Inc., San Diego, USA, 339 p

Hartmann, H.T., Kester, D.E. and Davies, Jr. F.T. 1983. Anatomical and physiological basis of propagation by cuttings. In: Plant propagation: Principles and practices. 5th Edition, Prentice Hall of India Pvt. Ltd., 242 p.

Hartmann, H.T., Kester, D.E., Davies, Jr. F.T. and Geneve, R.L. 2002. Principles of propagation by cuttings. In: Plant propagation: Principles and practices. 8th edition, Prentice Hall of India Pvt. Ltd., pp. 280-414

Kapulnik, Y., Okon, Y. and Henis, Y. 1985. Changes in root morphology of wheat caused by *Azospirillum* inoculation. *Canadian J. Microbiol.,* **31**:881-887

Lifshitz, R., Kloepper, J.W., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E.M. and Zaleska, I. 1987. Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Canadian J. Microbiol.,* **33**:390-395

Polat, A.A. and Kamiloglu, O. 2007. Experiment on propagation with cuttings of Quince-A andB A - 2 9 rootstocks and on budding with loquat cultivar. Turkey V. In: The proceedings of National Horticulture Congress, 4-7 September, Erzurum, Turkey, **1**:169-173

Rana, H.S., Rana, V.S. and Bhardwaj, D.R. 1999. Vegetative multiplication of kiwifruit (*Actinidia deliciosa* Chev.) through dormant cuttings. *Annal. For.,***7**:56-59

Siddiqui, M.I. and Hussain, S.A. 2007. Effect of indole butyric acid and types of cuttings on root initiation of *Ficus hawaii*. *Sarhad J. Agri.,* **23**:919-925

Singh, A., Patell, R.K., De, L.C. and Babu, K.D. 2008. Effect of cutting types, indole butyric acid and shoot pinching on rooting of kiwifruit (*Actinidia deliciosa*). *Indian J. Agril. Sci.,* **78**:695-698

Steenhoudt, O. and Vanderleyden, J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetics, biochemical and ecological aspect. *Microbiol. Rev.,* **24**:487-506

Taiz, L. and Zeiger, E. 2006. Plant Physiology, 4th edition, Sinauer Associates Inc. Publisher, Sunderland, Massachusetts, U.S.A., 286p

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