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EFFECTS OF IBA AND *TRICHODERMA* spp. ON ROOTING FEATURES OF *Rosa Centifolia* STEM CUTTINGS

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Abstract. The aim of this study was to evaluate the effect of different levels of (0, 1000, 2000 and 4000 ppm) IBA (indolyl-3-butyric acid), two fungi isolates, and their combinations on the rooting performance of *Rosa centifolia* cuttings. The application of IBA (with or without *Trichoderma* spp.) significantly improved the degree and percentage of rooting in cuttings. The results indicate that the rooting ability of cuttings differs significantly between the treatments. Considering the number of roots per rooted cutting and rooting percentage, highest results were obtained from the application of the 4000 IBA (23,63 and %98 respectively). The rooting performance of the cutting treated with solely biological agents' solution showed worse performance than the samples treated with IBA containing solutions.

Keywords: *Auxin, Propagation, Trichoderma spp., Rosa centifolia*.

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1. Introduction

An economically very important floriculture crop, Rose, belongs to family *Rosaceae*, which is consisted of about 125 genera and encompassing nearly 3500 species (Castilon *et al*., 2006; Senapati & Rout, 2008; Heinrichs, 2008). According to Krussmann, (1981) the genus *Rosa* is native to temperate regions of northern hemisphere, which includes Asia, Middle East, Northern parts of China, North America and Europe (Phillips & Rix, 1988; Ertter, 2001). The 30-million-year old rose fossils obtained from research in Asia, Europe and the United States prove that its history is as old as the history of human civilization (Chakraborty, 2005). There are various anecdotes about roses in the history of many cultures and civilizations. For example, Semiramis, queen of Assyria and in the Zoroastrian text Bundehesh, which described that hundred-petalled rose and dog rose to develop thorns when evil came in the world (Lehner & Lehner, 1960; Joret, 1892). In addition, in the Turkish mysticism culture, the prophet Mohammed and the rose are two valuable elements that are identified with each other (Joret, 1892). Another example is that Sultan Mehmed II, who conquered Istanbul, was portrayed as smelling of a rose.

The rose has great diversity in the world and is not only used for ornamental purposes but also in the food, cosmetics and the medicinal industry (Basim & Basim, 2003; Ozkan *et al*., 2004; Achuthan *et al*., 2003; Kaur *et al*., 2007; Mahmood *et al*., 1996). Some species of roses could be used in oil production because of their biochemical properties (Lu *et al*., 2003). Common rose oil producing species in the world are *R. damascena* Mill., *Rosa gallica* L., *R. centifolia* L. and *R. alba* L. (Rusanov *et al*., 2005; Tabaei-Aghdaei *et al*., 2007).

The using of rooted cuttings or grafting are the most common method of propagation of roses (Zieslin,1996). In addition, seeds are also used for the propagation of species and rootstock (Horn *et al*., 1992). Although these methods are widely used, they have several difficulties such as low multiplication rate and dependence on the season, the low ability of root formation (Rogers & Smith, 1992; Pati *et al*., 2006). Therefore, it is necessary to investigate new effective ways for rapid mass propagation of roses (Shabbir *et al*., 2009).

There are many environmental and physiological ingredients that affect root genesis, with exogenous treatments on cuttings being particularly important (Couvillon, 1998). The auxins are frequently used as synthetic compounds for root induction in rose. Different works have shown indirectly that auxin can manage the generation of adventitious roots in woody cuttings, nevertheless, the ideal concentration of auxin depend on species (Jarvis, 1986). The different combinations of indole acetic acid and indole butyric acid were used to obtain rooted cuttings of *R. multiflora*, *R. bourboriana* and *R. moschata* in the study of Bhujbal and Kale (1973). Some of the recent studies confirm that fungi and bacteria in several genera (*Trichoderma*, *Agrobacterium*, *Bacillus*, *Pseudomonas* etc.) induce root formation in stem cuttings (Hatta *et. al*., 1996; Rinallo, 1999; Ercisli *et. al*., 2004; Clouston *et al*., 2010).

Beneficial biocontrol agent relationships result in more effective improved growth (Schirawski & Perlin 2018; Stringlis *et al*., 2018). *Trichoderma* species fungi are well known for their ability to stimulate plant growth and development, as well as their capacity to increase the tolerance of plants to abiotic and biotic stress (Lopez-Bucio *et al*., 2015).

The present experiment was planned to expand the success rate of cutting by increasing rooting percentage through the application of different concentrations of plant growth regulators and *Trichoderma* strains and to observe their effects on the growth of cuttings.

2. Material and Method

The study was conducted at the Ataturk Central Horticultural Research Institute (ACHRI), Yalova, Turkey in 2018. One thousand semi-hardwood cuttings of *Rosa centifolia* species having three or four dormant buds were collected from uniform, healthy, and vigorous plants of the stock garden of ACHRI. Different concentration of indole butyric acid (IBA) and biological agents (BioA) were used, with different combinations of each. The as BioA, different densities of *Trichoderma harzianum* Rifai KRL-AG2 and one other local *Trichoderma* strain, DY, were used in this study. For spore production, *T. harzianum* was grown on potato dextrose agar medium (PDA) (Merck) for 7 days at 25 ^{o}C. Conidial densities in the suspension (1.2×10^{7} spores/mL) were determined by use of a hemocytometer under a light microscope. For the IBA treatment, 3 cm bases of cuttings were treated with solutions using the quick-dip method (10 sec). For the BioA treatment, 3 cm bases of cuttings were treated with solutions (20 min). For the combine treatment (IBA+BioA), firstly 3 cm bases of cuttings were treated with hormone solutions using the quick-dip method (10 sec) then waited in *Trichoderma* strain solutions (20 min). After treatments, cuttings were planted at a depth of 3-4 cm to the rooting medium consisting of a 2: 1 ratio of peat + perl, which was moistened and prepared one day before. During the rooting period, the ambient temperature was recorded hourly (Figure 1). Roots were observed after four week of culture. Twenty-five individual plants observed in each plot to obtain data on different rooting features. The rooting (%), root number per cutting, root fresh and dry weight (mg) were recorded for each treatment (Nasri *et al*., 2015). The experimental scheme was a randomized block design with four replications. All the statistical procedures for morphological traits were obtained using the SAS Institute Inc. JMP®.

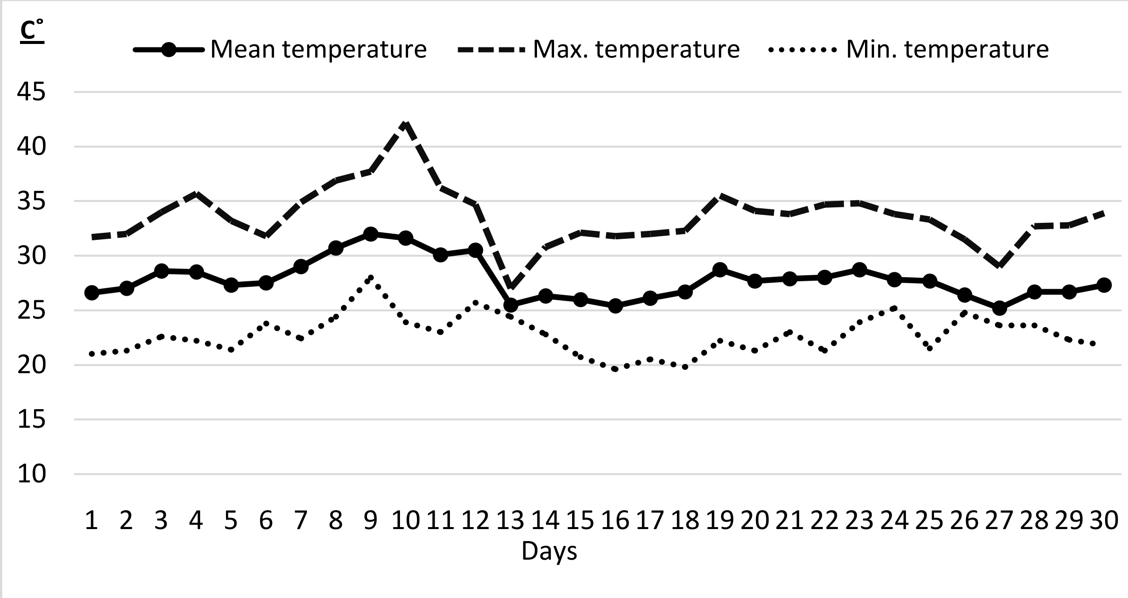

Figure 1. Ambient temperature of the greenhouse during the study

3. Results and Discussion

The effect of different IBA treatments and two biological agents (BioA): *Trichoderma harzianum* Rifai KRL-AG2 (T22) and DY, on measured traits in *Rosa centifolia* are presented in Table 1. The differences between the results were statistically significant in all observations ($p<0.1$). Among the treatments used, the highest rooting percents were recorded in IBA treatments without or with BioA. Among the treatments, the lowest rooting (85,00%) percent were recorded in the control treatment. It has been reported that auxin presence is essential for the initiation of the root starter cells (Hartman et al., 2002). The IBA effects on rooting were in accordance to the findings on *Rosa canina* (Kazankaya *et al*., (2005), on *Syzygium javanica* (Paul & Aditi, 2009), on *Rosa dumalis* (Ercisli *et al*., 2004), on *Rosa damescena* (Nasri *et al*., 2015).

The rapid immersion in auxin solutions might have supplemented the endogenous auxin ingredient at the base of cuttings. The reason for this might be the acceleration of the root initiation and genesis of root primordia that resulted in increased rooting in treated cuttings. It is assumed that the creation of adventitious roots in plants is controlled by growth substances and auxins are the principal hormones playing a direct role in this process (Gaspar & Hofinger, 1988).

Table 1. Effects of IBA and Biological Agents (T22 and DY) on rooting of rose cuttings

Treatments	Rooting percent	Fresh weight of roots (mg/plant)	Dry weight of roots (mg/plant)	Number of roots per rooted cutting
4000 ppm IBA	98,00 a	0,562 a	0,040 ab	23,63 a
2000 ppm IBA	97,00 a	0,630 a	0,048 a	20,41 ab
1000 ppm IBA	96,00 a	0,585 a	0,049 a	19,37 bcd
T22	88,00 bc	0,287 b	0,026 c	14,67 e
DY	82,00 c	0,330 b	0,028 bc	15,87 de
1000 ppm IBA + T22	94,00 ab	0,580 a	0,050 a	20,32 ab
2000 ppm IBA + T22	96,00 a	0,600 a	0,049 a	19,73 bc
1000 ppm IBA + DY	95,00 a	0,545 a	0,046 a	20,13 abc
2000 ppm IBA + DY	92,00 ab	0,545 a	0,044 a	21,06 ab
Control	85,00 c	0,307 b	0,029 bc	16,55 cde
P:	*<0,01*	*<0,01*	*<0,01*	*<0,01*
LSD:	*6,26*	*0,129*	*0,012*	*3,60*
CV (%):	*2,38*	*18,07*	*20,75*	*12,99*
F Ratios	*6,67*	*9,28*	*5,16*	*4,70*

Means in a column followed by the different letter are significantly different at the various levels as determined by LSD test. CV: coefficient of variation, LSD: least significance difference

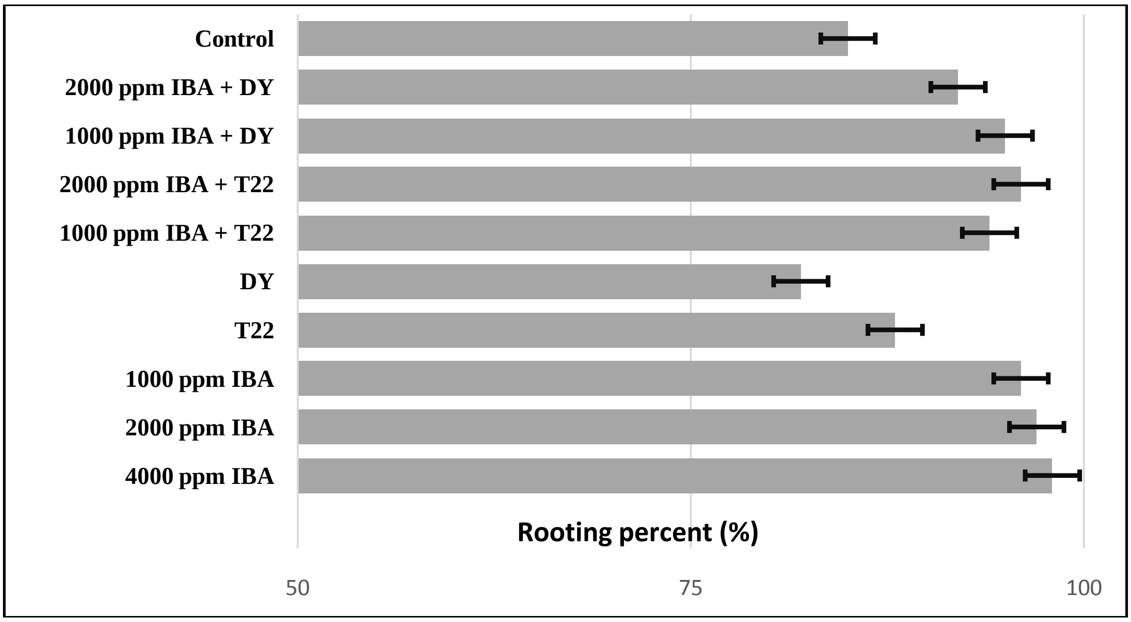

Figure 2. Percentage of root formation (%) in different treatments after two weeks of culture

Šebánek *et al.* (1991) reported that the generation of adventitious roots is involved in an enhancement in the level of auxin at the stem base. The meristematic actions of tissues can be controlled by Auxins. Also, it can enhance the supply of plastic substances at the sites of root formation. This leads to the widespread use of IBA as one of the stimulants that promote root formation. According to Hartmann *et al*. (2002) IBA application cause a high number of adventitious roots. IBA is the most efficient hormone for root formation of cuttings and it additionally accelerates the process (Mateja *et al*., 2005).

Results showed that the weights of fresh roots and weights of dry roots were significantly affected by IBA treatment. Among the tested treatments, the highest root fresh and dry weights (0,630 and 0,50 mg, respectively) were recorded in 2000 ppm IBA and 1000 ppm IBA + T22 application respectively. Among the treatments, the lowest root fresh weights (0,287 mg) were recorded in the application of T22. Similar results were reported by Al-Salem and Karam (2001) and Nasri *et. al.* (2015) on different rose rootstocks. The results of present study are similar to those of Ercisli and Guleryuz, (1999) and Ercisli *et. al.* (2004) indicating that hardwood stem cuttings of rosehip genotypes rooted best when treated with 2000 to 5000 ppm.

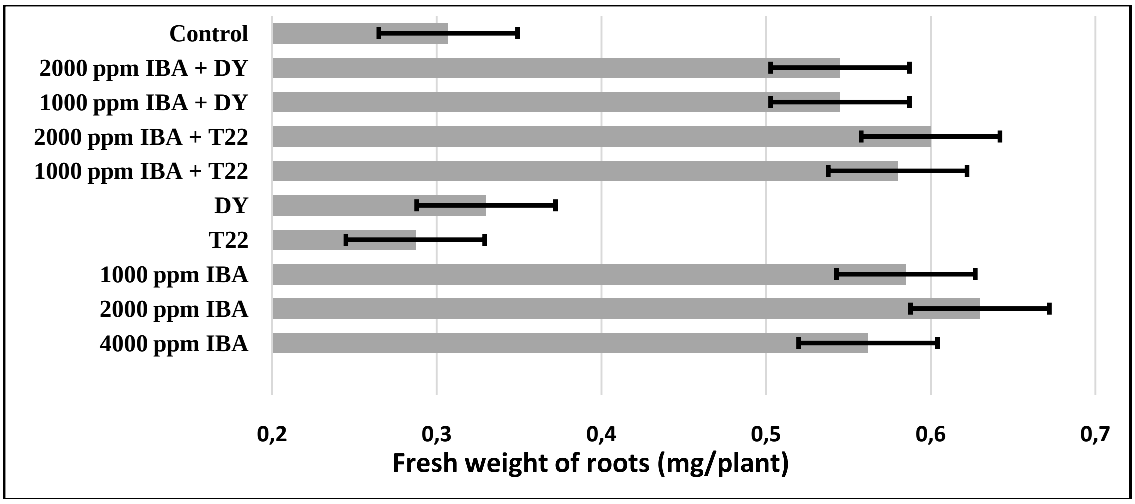

Figure 3. Percentage of fresh weights of rooted cuttings (mg/plant) in different treatments

Results showed that the number of roots was significantly affected by IBA treatment. Significant variations in root number in the evaluated treatments. The maximum number of roots (23, 63) was observed in 4000 ppm IBA, that showed a significant difference compared to the control (16,55) treatment. Also, treatments of T22 and DY without IBA were presented low root numbers (14,67 and 15,87, respectively). These results are in close conformity with findings of Akhtar *et al*. (2002) on *Rosa centifolia* and *Rosa damascene* and by Khan *et al*. (2004) on different rose rootstocks. Cuttings of rose treated with IBA rooted better than treated with biological agents. For example, rooting of cuttings was reduced when in oculated solely with T22 or DY without treatment with IBA. In addition, inoculation with *T22 or DY* strain was less effective than IBA or IBA+any kind of BioA considering the number of roots and root fresh weight. It is assumed that application Agrobacterium can induce adventitious rooting in recalcitrant woody genotypes (Bassil *et al*., 1991).

Our results were not in agreement with previously reported data (Bassil *et al*., 1991; Benavides, 1998; Esitken *et al*., 2003; Ercisli *et. al*., 2004), indicate that IBA-BioA combined treatments had more capability for increasing rooting of cuttings than IBA or bacteria alone treatments.

In the greenhouse where this study is carried out, the environmental conditions are controllable. As can be seen from Table 1, the ambient temperature has reached very high values in some periods.

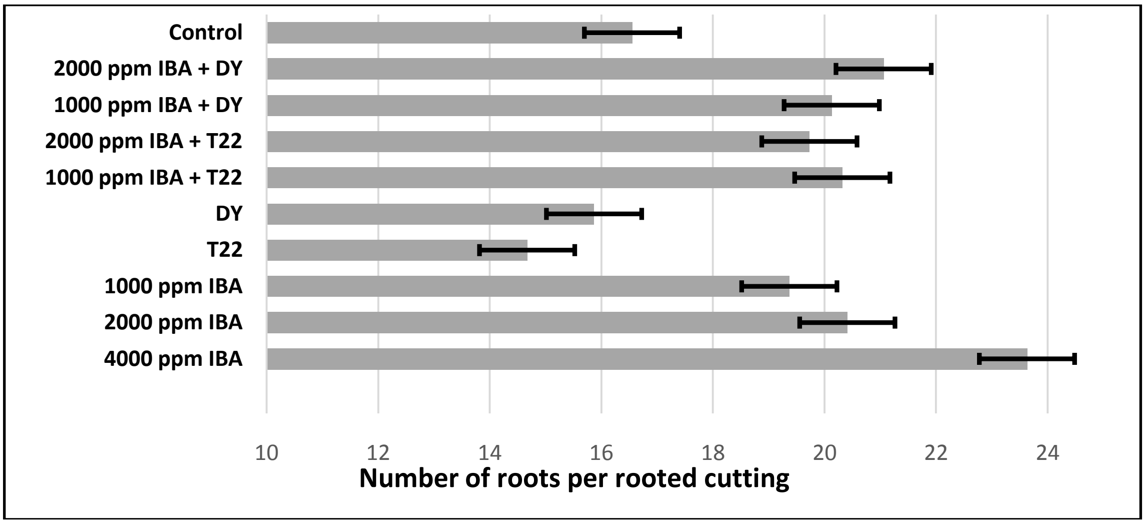

Figure 4. Percentage of dry weights of rooted cuttings (mg/plant) in different treatments

However, optimum conidial germination temperature of *Trichoderma* is reported as 15-30^{o}C. Similarly, the required temperature range for mycelial development is indicated as 15-30^{o}C, and the optimum temperature is 25^{o}C (Trutmann & Keane, 1990; Santamarina & Roselló, 2006; Jackson *et al*., 1991). According to the results obtained, it can be concluded that the high temperature in the experimental environment reduces the activities of biological agents.

References

Achuthan, C.R., Babu B.H., Padikkala J. (2003). Antioxidant and hepatoprotective effects of Rosa damascene. *Pharmaceutical Biology oil. Fitoterapia*, 74, 394-396.

Akhtar, M.S., Khan, M.A., Atif-Riaz, Adnan-Younis, (2002). Response of different rose species to different root promoting hormones. *Pakistan Journal of Agricultural Sciences,* 39, 297-299.

Al-Salem, M.M., Karam, N.S. (2001). Auxin, wounding, and propagation medium affectrooting response of stem cuttings of Arbutus andrachne. *Hort Science*, 36, 976-978.

Bassil, N.V, Proebsting, W.M., Moore, L.W., Lightfoot, D.A. (1991). Propagation of hazelnut stem cuttings using Agrobacterium rhizogenes. *Hort. Science,* 26, 1058–1060.

Basim, E., Basim H. (2003). Antibacterial activity of Rosa damascene essential oil. *Elsevier Science, Fitoterapia*, 74, 394-396.

Benavides, M.P., Radice S. (1998). Root induc- tion in Simmondsiachiensis (Link) Schneid. using Agrobacterium rhizogenes. *Biocell,* 22, 109–114.

Bhujbal, B.G, Kale, P.N. (1973). Effect of some growth regulators on rooting of cutting of different rootstocks of rose (Rosa sp.). *Punjab Hort*., 13, 50-53.

Castilon, J., Jones B., Kamo K. (2006). Efficient regeneration of rose plants from somatic embryos of three genetically diverse cultivars. *Floral Nursery Plants Research Unit, US National Arboretum*, Beltsville.

Clouston, A.M., Hill, R.A., Minchin, R., Braithwaite, M., & Stewart, A. (2010). A bioassay screening Trichoderma isolates for enhancement of root development in Impatiens walleriana cuttings. *New Zealand plant protection*, *63,* 33-38.

Couvillon, G.A. (1998). Rooting Responses to Different Treatments. *Acta Hort*., 227, 187-196.

Ercisli, S., Guleryuz M. (1999). A study of the propagation of the hardwood cuttings of some rose hips. *Tr. J. Agr. For*., 23 (Suppl. 2), 305–310.

Ercisli S., Esitken, A., Sahin, F. (2004). Exogenous IBAand Inoculation with Agrobacterium rubi Stimulate Adventitious Root Formation on Hardwood Stem Cuttings of Two Rose Genotypes, *Hortscience*, 39(3).

Ertter, B. (2001). Native California Roses. An on-line Monograph published through the University of California, available online at http://ucjeps.Berkeley.edu/ina/roses/roses:html

Chakraborty, K. (2005). Knowing Roses. *The Rose Bulletin*, 4-5.

Eşitken, A., Ercişli, S., Şevik, İ., & Şahin, F. (2003). Effect of indole-3-butyric acid and different strains of Agrobacterium rubi on adventive root formation from softwood and semi-hardwood wild sour cherry cuttings. *Turkish Journal of Agriculture and Forestry*, *27*(1), 37-42.

Gaspar, T., Hofinger, M. (1988). Auxin Metabolism During Adventitious rooting. In: Davis, T.D., Haissig, B.E., Sankhla, N. (eds.), Adventitious Root Formation in Cuttings. *Portland*, Dioscorides Press, 117-131.

Hartmann, H.T., Kester, D.E., Davies, F.T., Geneve, R.L. (2002), *Plant Propagation: Principles and Practices*, Prentice Hall, New Delhi, India.

Hatta, M, Beyl, C.A., Garton, S., Diner, A.M. (1996). Induction of roots on jujube softwood cuttings using Agrobacterium rhizogenus. *J. Hort. Sci*., 71, 881–886.

Heinrichs, F. (2008). International statistics flowers and plants, International Association of Horticultural Producers/Union Fleurs, Voorhout, The Netherlands, 56, 16-90

Horn WAH. (1992). Micropropagation of rose (*Rosa* spp. L.). In Bajaj, Y.P.S. (ed.), *Biotechnology in Agriculture and Forestry*, 20, 320-342.

Jackson, A.M., Whipps, J.M., Lynch, J.M. (1991) Effects of temperature, pH and water potential on growth of four fungi with disease biocontrol potential. *World Journal of Microbiology and Biotechnology*. Oxford, 7(9), 494-501.

Jarvis, B.C. (1988). Adventitious root formation with respect to auxin distribution. In *Physiology and biochemistry of auxins in plants: proceedings of the symposium, held at Liblice, Czechoslovakia, September 28-October 2, 1987/edited by Milan Kutacek, Robert S. Bandurski and Jan Krekule*. Prague, Czechoslovakia: Academia.

Joret, C. (1892). *La Rose dans l antiquite et au moyen age*. Emile Bouillon, Paris.

Kaur, N., Sharma, R.K., Sharma, M., Singh, V., Ahuja, P.S. (2007). Molecular evaluation and micropropagation of field selected elites of R. damascena. *General and Applied Plant Physiology*, 33, 171-186.

Kazankaya, A., Yoruk, E., Dogan, A. (2005). Effect of IBA on rooting of Rosa canina hardwood cuttings from Lake Van Region, Turkey. *Acta Horticulturae*, 690, 153-158.

Khan, M.A., Khurram, Z., Iftikhar, A. (2004). Effect of various hormones and different rootstocks on rose propagation. *Pakistan Journal of Biological Sciences*, 7, 1643-1646.

Krussmann, G. (1981). The complete book of roses. Portland, Oregon: Timber Press Lehner, E., and J. Lehner, 1960. Folklore and Symbolism of Flowers, *Plants and Trees*. Tudor, New York.

Lo´pez-Bucio, J., Pelagio-Flores, R., Herrera-Estrella, A. (2015). Trichoderma as biostimulant: Exploiting the multilevel properties of a plant beneficial fungus. *Sci. Hortic. (Amsterdam)* 196, 109-123.

Lu, L., Gu, C., Li, C., Alexander, C., Bartholomew, B., Brach, A.R., Boufford, D.E., Ikeda, H., Ohba, H., Robertson, K.R. (2003). *Spongberg. Rosaceae*. Flora of China, 9, 46-434.

Mahmood, N., Piacente S., Pizza C., Burke A., Khan A.I., Hay A.J. (1996). The Anti-HIV activity and mechanisms of action of pure compounds isolated from *Rosa damascene*. *Biochemical and Biophysical Research Communications*. 229, 73-79.

Mateja, S., Dominik, V., Franci, S., Gregor, O. (2007). The effects of a fogging system on the physiological status and rooting capacity of leafy cuttings of woody species. *Trees,* 21, 491–496.

Nasri, F., Fadakar, A., Saba, M.K., Yousefi, B. (2015). Study of Indole Butyric Acid (IBA) Effects on Cutting Rooting Improving Some of Wild Genotypes of Damask Roses (Rosa damascena Mill.). *Journal of Agricultural Sciences*, 60(3), 263-275.

Ozkan, G., Sagdic, O., Baydarand, N.G., Baydar, H. (2004). Antioxidant and antibacterial activities of Rosa damascene flower extracts. *Food Science and Technology,* 10, 277-281.

Pati, P.K., Rath, S.P., Sharma, M., Soodand, A., Ahuja, P.S. (2006). In vitro propagation of rose: A review. *Biotechnol. Adv*., 24, 94-114.

Paul, R., Aditi, C. (2009). IBA and NAA of 1000 ppm induce more improved rooting characters in air-layers of waterapple (Syzygiumjavanica L.). *Bulgarian Journal of Agricultural Science,* 15, 123-128.

Phillips, R., Rix, M. (1988). *Roses.* Pan Books Ltd, London.

Rinallo, C., Mittempergher, L., Frugis, G., Mariotti, D. (1999). Clonal propagation in the genus Ulmus: Improvement of rooting ability by Agrobacterium rhizogenes T-DNA genes. *J. Hort. Sci. Biotech*., 74(4), 502-506.

Rogers, R.B., Smith, M.A.L. (1992). Consequence of in vitro and ex vitroroot initiation for miniature rose production. *J. Hort. Sci*., 67, 535-540.

Rusanov, K., Kovacheva, N., Vosman, B., Zhang, L., Rajapakse, S., Atanassov, A., Atanassov, I. (2005). Microsatellite analysis of Rosa damascenaMill. accessions revealsgenetic similarity between genotypes used for rose oil production and old Damask rose varieties. *Theor. Appl. Genet*., 111, 804-809.

Santamarina, M.P., Roselló, J. (2006) Influence of temperature and water activity on the antagonism of *Trichoderma harzianum* to *Verticillium* and *Rhizoctonia*. *Crop Protection*. Guildford, 25(10), 1130–1134.

Schirawski, J., Perlin, M. (2018). Plant-Microbe Interaction 2017-The good, the bad and the diverse. *Int. J. Mol. Sci*., 19, 1374.

Senapati, S.K., Rout, G.R. (2008). Study of culture conditions for improved micropropagation of hybrid rose. *Hort. Sci. (Prague),* 35(1), 27-34.

Shabbir, A., Hameed, N., Ali, A., Bajwa, R. (2009). Effect of different cultural conditions on micropropagation of rose (Rosa indica L.). *Pak. J. Bot.,* 41, 2877-2882.

Stringlis, I.A., Zhang, H., Pieterse, C.M.J., Bolton, M.D., de Jonge, R. (2018). Microbial small molecules-weapons of plant subversion. *Nat. Prod. Rep*., 35, 410-433.

Šebánek, J., Sladký, Z., Procházka, S. (1991). Experimental morphogenesis and integration of plants, *Development in crop science*, 18, 180-184.

Tabaei-Aghdaei, S.R., Babaei, A., Khosh-Khui, M., Jaimand, K., Rezaee, M.B., Assarehand, M.H., Naghavi, M.R. (2007). Morphological and oil content variations amongst Damask rose (*Rosa damascene* Mill.) landraces from different regions of Iran. *Sci. Hort.*, 113, 44-48.

Trutmann, P., Keane, P.J. (1990) *Trichoderma konigii* as a biological control agent for *Sclerotinia sclerotiorum* in Southern Australia. *Soil Biology & Biochemistry*, 22(1), 43-50.

Zieslin, N. (1996). Influence of climatic and socio-economical factors on mode of cultivation and research of rose plants. *Acta Hortic*., 424, 21-22.