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EFFECT OF BIOTIZATION ON GRAFTING SUCCESS OF THE PLANTS IN FRUIT NURSERY

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Abstract- One of the most important agrotechnical manipulations in the nurseries is grafting of the fruit tree varieties on the suitable rootstocks. The objective of our research was to observe the effect of different biotizations on grafting success of the plants in the fruit nursery. OHF 33 rootstock grafted with pear cultivar William displayed the best grafting rates reaching up to 61,40% in *Glomus intraradices*+ *Trichoderma harzianum* T22 variant. For comparison, the control variants in OHF 33 experimental plots showed grafting success values between 33,64 % and 40,60%. T-budding grafting of Myrobolan 29 were remarkably unsuccessful, showing 11,0 % as a best rate (again in *Glomus intraradices*+ *Trichoderma harzianum* T22 variant). In this case, except strong winter temperatures also graft incompatibility with plum cultivar Angelino may appear as a limited factor having in mind comparison with other rootstock/scion combinations. There are not observed statistically distinguishable differences between mycorrhizated and non-mycorrhizated variants inside of the GF 677/ Royal Glory experimental plots. In case of Gisela 6/cv. Van rootstock/scion combination we observed a successful grafting rate ranging from 25,67% (var. *Glomus intraradices*) to 44,44% (var. *Trichoderma harzianum* T22+ *Baccilus subtilis*). Best rates, in general, are obtained for *Trichoderma harzianum* T22 biotizated plants, which overrun the controls with 3% to 11%.

Keywords- Fruit nursery, Grafting, Mycorrhiza, *Streptomyces lydicus*, *Bacillus subtilis*, *Agrobacterium radiobacter*

Introduction

Vegetative propagation of fruit species traditionally occurs by cuttings or grafting. In the latter case, farmers use suitable rootstocks to acquire tolerance for different types of abiotic and biotic stress, keeping the desired characteristics of the cultivar in the scion [1].

One of the most important agrotechnical manipulations in the nurseries is grafting of the fruit tree varieties on the suitable rootstocks [2]. When grafting is made in practice, we usually depend on our experience, and thus fundamental and important problems on grafting remain unsolved, especially with reference to the mechanism of grafting [3]. The first stage of the fusion in grafting begins with the contact of calluses which are formed on the cut surfaces of rootstocks and scions. These contacted calluses maintain cell division and proliferation, and consequently they tightly unite. As the second stage, the supply of nutrition and water through the calluses from rootstock to scion gives rise to the elongation of new shoots and the growth in thickness near the joint part. In this way, the fusion of rootstock and scion is completed [4]. Other important factors for successful grafting rate are air and soil temperatures, humidity and fertilization [5]. In general, the environmental factors have more influence over the grafting success in the countries with temperate climate (as Bulgaria), having in mind the strong lowering winter temperatures during January and February [6].

T-Budding is a preferable grafting method in most of the fruit growing species, providing better success rate and plant development. T -budding is performed when the rootstock bark is slipping during the early summer and the scion buds have matured and hardened [7] [Fig-1].

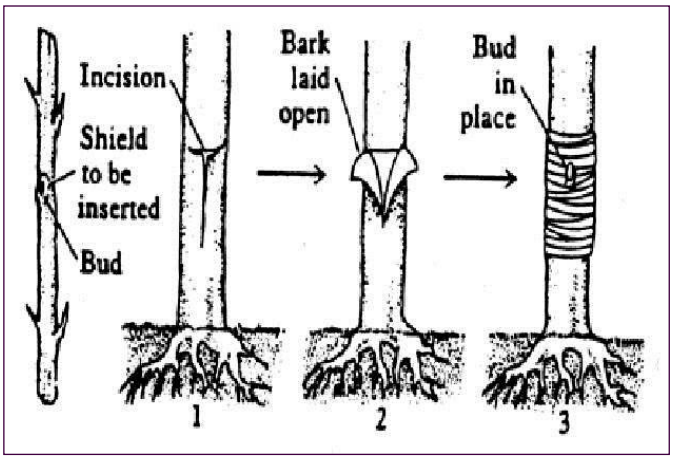

Fig. 1- The T-bud is the most common method used to bud fruit-growing variety scion onto rootstock. The shield is cut from the budstick and inserted into a T-cut on the stock.

Mycorrhizal fungi have been shown to increase plant uptake of water and mineral nutrient [8]. Until now, the influence of mycorrhizae inoculation on the grafting/budding success is not clarified [9]. The objective of our research was to observe the effect of different biotizations on grafting success of the plants in the fruit nursery.

Materials and Methods

In vitro obtained and biotizated rootstocks were produced by SME Fitotechniki- Greece. They were planted in the Bulgarian project experimental field (Sliven region) on 16th of April 2009. Sliven is

located 300 km east of Bulgaria's capital Sofia. Sliven Municipality is situated on the sub-Balkan plain in the zone of transitional-continental climate. West of the city lies the so-called 'Peach' Valley, where large peach orchards are planted. The project experimental field is 5 km from the town (nearby Krushare village). The soil samples of experimental field have a pH neutral up to low alkali with low humus percentage (according to organic content classification scale). Soil nutrition is not well balanced for basic elements and the soil is within the confines of very low supplied ones. The content of microelements is in the frame of average for this soil type. From the other side, the soil has a light mechanical composition with content of physical clay in plough part. Regarding to microbiological analyses, the samples are characterized with comparatively low micro biogenesis. Quantity of microorganism is decreasing proportionally to the increasing of depth of soil profile that is a result of poor soil aeration and nutrition conditions. The low content of microscopic fungi probably is a result of the non- suitable soil conditions for their development. There were not found any microorganisms from the species *Trichoderma* spp., *Streptomyces* spp. and *Agrobacterium radiobacter* in the initial soil samples from experimental field. Distribution of mycorrizated plants were done on experimental plots, related to the project work plan (distances: 1,2 x 0,2 m / 1,0 m between the plots). Additional treatments with bio-agents *Baccilus subtilis* and *Streptomyces lydicus* in both *Trichoderma harzianum* T22 and Control SITINPLANT plots was made on 25th of May and 25th of July 2009 respectively.

The rootstocks were grafted with the cultivar scions on 14-15 and 16th of August 2009 through T-budding, 10 cm from the soil level, with one- bud scions. In the spring of the following year, the rootstock part situated above the bud was cut off. The percentage of successfully grafted plants was observed and calculated on 15th of April 2010.

Results and Discussions

Fruit rootstocks are usually propagated by seeds or by cuttings. Propagation by seeds can generate a large amount of genetic variation in the rootstocks, affecting grafting efficiency and plant yield in the field [10]. On the other hand, cuttings harvested from field plants can also reduce the efficiency of the grafting process and can spread diseases into the new plantations [11]. It is evident, for both the industry and breeders, that tissue culture has the potential to produce rootstocks on a large scale faster than the traditional methods. Additionally, this technology can guarantee the production of genetically identical, physiologically uniform and pathogen-free plants at reasonable costs [12-14]. Related with the above, in our case, *in vitro* obtained and biotized four different rootstocks presented a good prerequisite for production of quality fruit plants in the nursery. The summarized results of the grafting success are presented in [Table-1].

Table 1- Effect of biotizations on grafting success of the experimental plants

Rootstock	Experimental Plot/Treatment	Grafted Plants (No.)	Grafting Success (%)	Grafted Variety
	1 - *Trichoderma harzianum* T22	180	41,66	
	1 - *Trichoderma harzianum* T22+ *Bacillus subtilis*	180	44,44	
	1 - *Trichoderma harzianum* T22+ *Streptomyces lydicus*	180	43,33	
	2 - *Glomus intraradices*	300	25,67	
GISELA 6	3 - Control SITINPLANT	180	37,78	cv.Van
	3 - Control SITINPLANT+ *Bacillus subtilis*	180	37,22	
	3 - Control SITINPLANT+ *Streptomyces lydicus*	180	35,56	
	4 - *Glomus intraradices*+ *Trichoderma harzianum* T22	150	36,67	
	5 - Control Nursery	220	33,64	
	1 - *Trichoderma harzianum* T22	165	41,21	
	1 - *Trichoderma harzianum* T22+ *Bacillus subtilis*	165	43,64	
	1 - *Trichoderma harzianum* T22+ *Streptomyces lydicus*	165	46,06	
	2 - *Glomus intraradices*	220	34,09	
OHF 33	3 - Control SITINPLANT	165	38,79	cv. Willam
	3 - Control SITINPLANT+ *Bacillus subtilis*	165	40,60	
	3 - Control SITINPLANT+ *Streptomyces lydicus*	165	38,79	
	4 - *Glomus intraradices*+ *Trichoderma harzianum* T22	114	61,40	
	5 - Control Nursery	220	33,64	
	1 - *Trichoderma harzianum* T22	200	6,0	
	1 - *Trichoderma harzianum* T22+ *Bacillus subtilis*	200	7,5	
	1 - *Trichoderma harzianum* T22+ *Streptomyces lydicus*	200	7,5	
	2 - *Glomus intraradices*	300	4,33	
Myrobolan 29 C	3 - Control SITINPLANT	165	8,48	cv. Angelino
	3 - Control SITINPLANT+ *Bacillus subtilis*	165	9,09	
	3 - Control SITINPLANT+ *Streptomyces lydicus*	165	6,06	
	4 - *Glomus intraradices*+ *Trichoderma harzianum* T22	100	11,0	
	5 - Control Nursery	370	2,43	
	1 - *Trichoderma harzianum* T22	315	23,81	
	1 - *Trichoderma harzianum* T22+ *Bacillus subtilis*	315	24,76	
	1 - *Trichoderma harzianum* T22+ *Streptomyces lydicus*	315	23,17	
	2 - *Glomus intraradices*	220	23,64	
GF 677	3 - Control SITINPLANT	185	23,78	cv. Royal Glory
	3 - Control SITINPLANT+ *Bacillus subtilis*	185	25,94	
	3 - Control SITINPLANT+ *Streptomyces lydicus*	185	16,21	
	4 - *Glomus intraradices*+ *Agrobacterium radiobacter* K84	300	20,0	
	5 - Control Nursery	700	7,85	

Gisela 6 Grafted with Cherry Cultivar Van

In this rootstock/scion combination we observed a successful grafting rate in percentage between 25, 67% (var. *Glomus intraradices*) and 44, 44% (var. *Trichoderma harzianum* T22+ *Baccilus subtilis*) regarding low and high values. There are not statistically significant differences into the *Trichoderma harzianum* T22 variants. The same trend showed Control SITINPLANT plots, which mean that in case of the rootstocks/scion combination we do not find any positive or negative effect of the additional treatments with bio agents *Bacillus subtilis* and *Streptomyces lydicus* over grafting success. Best rates, in general, are observed among *Trichoderma harzianum* T22 biotizated plants which overrun the controls with values between 3% and 11% [Fig-2].

Fig. 2- Gisela 6/cv. Van T-budding grafted experimental plants

OHF 33 Grafted with Pear Cultivar William

Compared with the obtained results for other experimental rootstocks, here we have the best rates reaching up to 61,40% in *Glomus intraradices* + *Trichoderma harzianum* T22 variant. The control ones showed grafting success values between 33, 64% and 40,60%.

As in the previous rootstock, there are not statistically significant differences in cases of additional bacterial treatments in *Trichoderma harzianum* T22 and Control SITINPLANT plots.

Myrobolan 29 C Grafted with Plum Cultivar Angelino

T-budding grafting in this rootstock was remarkably unsuccessful, showing 11,0 % as a best rate (*Glomus intraradices* + *Trichoderma harzianum* T22 variant). In this case, except strong winter temperatures also graft incompatibility might become a limited factor, having in mind comparison with other rootstock/scion combinations. It is necessary to continue additional grafting experiments with other plum or apricot varieties.

GF 677 Grafted with Peach Variety Royal Glory

There are no remarkable differences between mycorrhizated and non-mycorrhizated variants inside the SITINPLANT plots, the percentage data vary between 23,17% and 25,94%. Exceptions are *Glomus* + *Agrobacterium* combination (20,0 %) and Control SITINPLANT + *Streptomyces lydicus* (16,21%). Lowest grafting success was observed in Control Nursery plants (7,85%) [Fig-3].

Fig. 3- Successful and non-successful (frozen) T-budding grafted plants GF 677/cv. Royal Glory

Unsuccessful grafting depends on callus formation during the graft union and the failure of either cambial differentiation or incomplete steps during this period [15]. These incomplete steps could result from a technically incorrect graft, time of the year, incompatibility between rootstock and scion, as well as the environmental factors such as temperature and moisture [16-18]. In general, anatomical grafting incompatibility is a result of failure of the union of cambium tissues of stock with scion to establish callus formation. In case of utilizing different rootstocks and scions species, graft incompatibility is much more noticeable and arise clearly due to anatomical, physiological and biochemical differentiation and their interaction [19]. Concerning the current study, possible hypothesis is that the most limited factor in our case was untypical extremely strong environmental conditions in the experimental field during 2009/2010 winter (temperature below -20°C and strong cold wind at the same time). That leaded to low snow coverage (less than 2 cm) and increased the possibilities for frozen damages of the grafted buds, even when the T-budding was preventively made on 10 cm distance from the soil level. In case of Myrobolan 29, graft incompatibility or type of grafting could be additional limiting factor. Unsuccessful graft would be determined by anatomical or histological examination at early stage, since cambium develops within 6 to 8 weeks [20]. However, this kind of evaluation would give us information only about anatomical incompatibility. A more deep research on physiological and biochemical differences is needed. Further field experiments of different rootstock/scion combinations would also be very useful.

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Conflicts of Interest: None declared.

References

[1] Carrasco B., Meisel L., Gebauer M., Garcia- Gonzales R. and Silva H. (2013) *Biol. Res*., 46, 219-230.

[2] Feucht W. (1988) *Acta Hort.,* 227, 33-42.

[3] Fudjii T. and Nito N. (1972) *J. Japan. Soc. Hort. Sci.,* 41 (1), 1-10.

[4] Moore R. (1984) *Amer. J. Bot.,* 71 (5), 752-758.

[5] Litchev V., Gurnevsky V., Stoyanov A. and Tabakov S. (2003) *Pomology*, Agricultural University, 186-189.

[6] Gandev S. and Dzhuvinov V. (2005) *Acta Hort., (ISHS),* 705, 351-353.

[7] Hartmann H.T., Kester D.E., Davies F.T. and Geneve R.L. (1997) *Plant Propagation: Principles and Practices*, 6th ed., Prentic Hall, 770-790.

[8] Maronek D., Hendrix J. and Keirnan J. (1982) *Hort. Rev.,* 3, 172 -213.

[9] GTZ-ITFSP (2008) *Tree Crop Propagation and Management - A Farmer-Trainer Training Manual*, 5-10.

[10] Bouhadida M., Gonzalo M., Moreno M., Arus P., Casas A. and Gogorcena Y. (2009) *Sci. Hort.*, 120, 237-245.

[11] Vujovic T., Ruzic D. and Cerovic R. (2012) *Hort. Sci.*, 39, 101-107.

[12] Garcia-Gonzales R., Quiroz K., Caligari P. and Carrasco B. (2010) *Rev. Cs. Inv. Agr.*, 37, 5-30.

[13] Isac V., Coman T., Marinescu L., Isac M., Teodorescu A., Popescu A., Coman M. and Plopa C. (2010) *Rom. Biotech. Lett.*, 15, 92-101.

[14] Balla I. and Mansvelt L. (2013) *Protocols for Micropropagation of Selected Economically-Important Horticultural Plants*, 137-148.

[15] Moore R. (1984) *American Journal of Botany*, 752-758.

[16] Chalise B., Baral D., Gautam D. and Thapa R. (2013) *Nepal Journal of Science and Technology*, 14(1), 23-30.

[17] Mir M. and Kumar A. (2011) *International Journal of Farm Sciences*, 1(2), 17-22.

[18] Singh R., Karuna K., Kumar A. and Mankar A. (2012) *Progressive Horticulture*, 44(1), 153-156.

[19] Errea P. (1998) *Scientia Horticulturae*, 74(3), 195-205.

[20] Pina A. and Errea P. (2005) *Scientia Horticulturae*, 106(1), 1-11.