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BIOSTIMULANT APPLICATION IN TRANSPLANTS PRODUCTION *OF Allium Sativum* L. AND *WILD ROSES* (*Rosa Canina* L.)

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Abstract

Advantages of *in vitro* propagation of some vegetable and flower transplants is that can be started with very little amount of plant material that represents initial explants, which is very important in the protection of endangered and rare species. Slavonian winter garlic is an old, indigenous variety of garlic grown on Slavonia and Baranya fields, east Croatia. Today, unfortunately, due to the introduction of foreign varieties into production, lack of local seed source, the increasing commercialization of vegetable production and the depopulation of rural areas, large part of the local varieties of garlic may be lost forever. Method of *in vitro* propagation provides a rapid propagation and large number of plants as a single mother plant can produce up to several thousand of seedlings. Aim of this study was to investigate the influence of biostimulant on adaptation of garlic and wild rose in vitro seedlings. *In vitro* transplants of *Allium sativum* L. and *Rosa canina* L. were transplanted in commercial substrate and treated with biostimulant Radifarm® by watering. Influence of biostimulant had positive effect on shoot number and root weight. Investigation shows how biostimulant application in *Rosa canina* and *Allium sativum* transplants production improves growth and development of root and above-ground mass which is important for faster plant adaptation on stress during transplanting.

Key words: *biostimulant, winter slavonian garlic, wild roses, in vitro propagation*

Introduction

In vitro culture of plant cells, tissues and organs is a special branch of plant biotechnology, which is a set of techniques for sterile breeding and vegetative propagation of plants, plant organs, tissues and cells in a defined nutrient medium and under controlled environmental conditions. Largest application of tissue culture methods is in the reproduction of many plant species, especially those that are difficult to reproduce by seeds. Advantages of *in vitro* propagation is that the propagation can start with very little amount of plant material that represents initial explants, which is very important in the protection of endangered and rare species. Slavonian winter garlic is an old, indigenous variety of garlic grown on Slavonia and Baranya fields. Today, unfortunately, due to the introduction of foreign varieties into production, lack of local seed source, the increasing commercialization of vegetable production and the depopulation of rural areas, large part of the local varieties of garlic may be lost forever. *In vitro* propagation of garlic has been reported in many studies using a variety of micropropagation methods (Nagakubo *et al*., 1993; Khan *et al*., 2004; Ayabe and Sumi, 1998).

The usage of *Rosa canina* plant is various and therefore it found its place in culinary, decorating, cosmetic industry, horticulture and as medicinal plant. The most commonly used part of the plant for its medicinal properties is the fruit which are well known to contain a large amount of vitamin C (Daels-Rakotoarison *et al*., 2002; Kilicgun *et al*., 2009). Thus it is considered to be an astringent, carminative, diuretic, laxative, ophthalmic and tonic. Besides being used for its medicinal properties, wild roses found a place in floriculture as rootstock for grafting or breeding of cultivated species of roses. The propagation of roses can be performed with difficulties caused by poor germination of seeds (if they reproduce generative) or poor rooting of cuttings (if they reproduce vegetative) (Šindrek et al., 2013). A modern method of tissue culture provides faster reproduction of wild roses rootstocks and thus shortened breeding time to one year. After successful propagation of *in vitro* seedlings, the problem can occur in adaptation phase when only 50% of seedlings survives. Application of biostimulants can influence on better root development of seedlings which contributes to more successful adaptation with minimal losses. Biostimulants which contains glycosides, amino acids, arginine and asparagine promotes root hair growth and root function in higher plants (Garcia *et al*., 2006). Research by Zeljković *et al*. (2010) confirmed that application of biostimulant compared to untreated plants of *Salvia splendens* L. transplants immediately after transplanting positively affects plant growth and development. Furthermore, it has been reported that *Tagetes patula* L. transplants treated with biostimulants had improved root and above-ground part growth (Parađiković *et al*., 2009). Thus, the aim of this study was to investigate the influence of biostimulant on adaptation of garlic and wild rose *in vitro* seedlings.

Materials and methods

Plant Material and Sterilization

Rosa canina plant material have been collected from the rural area of Slavonia - Baranya County, whereas cloves of Slavonian winter garlic was taken from personal production in July 2013. The plant material of both species was firstly washed with running tap water. After cutting, nodes of wild rose stem and peeled garlic cloves were washed again under tap water followed by addition of two drops of liquid soap for about 15 minutes. Sterilization was conducted by immersing the prepared garlic and wild rose plant material in a series of solutions: initially with 70% ethanol for 1 min, then by 10 % bleach, with a drop of detergent solution for 20-30 min and then again 70% ethanol for 1 min and finally washed five times with sterile distilled water. During the course of the sterilization treatments, the plants were periodically stirred.

Culture media and conditions for shoot regeneration

The basal medium consisted of MS mineral salts and organics (Murashige and Skoog, 1962) supplemented with 30 g sucrose and solidified with 6.4 g agar powder. The pH was adjusted to 5.8 prior to autoclaving at 121°C with a pressure of 1.5 bar for 20 min. For experimental trials, the basal medium was supplemented with 1 mgl^{-1} Indole-3-butyric acid (IBA) and 5 mgl^{-1} 6-benzylaminopurine (BAP). Cultures were incubated at 20°C for 30 days under a 16-h light/8-h dark photoperiod. Light was supplied by cool white fluorescent lamps (20–30 $molm^2$ s^{-1}).

Elongation and rooting

After 4 weeks of culture, garlic and wild rose explants with regenerated shoots were transferred to fresh MS medium containing the same concentrations and types of plant growth regulators supplemented with NAA (5 mgl^{-1}) and cultured under light conditions for shoot elongation and rooting. The number of normal elongated and rooted shoots was recorded after 4 weeks of growth.

Acclimatization of regenerated plants

Regenerated plants with well-developed roots were removed from culture bottles, washed free of agar with warm water and transferred to container containing commercial substrate (Fruhstorfer Erde, Premium Blumenerde).

Acclimatization of plants was observed in growth chamber for 4 weeks at 20°C with 12-h light regime.
Forty plants of wild rose and forty plants of garlic were transplanted in four repetitions representing treatment and control. Treatment with biostimulant Radifarm® (Valagro SpA, Italy) in the concentration of 0.25% was carried out immediately after the transplantation and each following week. Radifarm® belongs to a group of biostimulants containing glucosides (energy growth factors) and amino-acids (arginine and asparagine) which stimulate root development. After four weeks the transplants were transferred to small pots containing the same substrates where its development and Radifarm® treatment was continued. When the wild rose plants were sufficiently developed for permanent transplantation, root length and mass were recorded. Garlic plants were grown till end of the vegetation when they were harvested and following parameters were recorded: total plant mass, bulb mass and number of cloves per bulb.

Results and discussion

The treatment with biostimulant showed a significant difference of wild rose root mass compared to control plants (p = 0.01). The greatest root mass was recorded on plants treated with the biostimulant (21.010 g), while the smallest root mass was recorded on control plants (15.670 g). Plants treated with biostimulant had 13.96% greater root mass than control plants. Similar results were presented by Vinković *et al.* (2009) in whose research the largest root mass of tomato were recorded in plants treated with biostimulant Radifarm ®.
Root length was not significantly influenced by biostimulant (Table 1).

Table 1. Influence of biostimulant on root length and fresh mass of wild rose

Wild rose	Control	Treatment	Mean
Root length	**19,045**	**19,800**	**19,422**
Root mass	**18,163^{b}**	**21,010^{a}**	**19,586**
	Root length		
LSD	Radifarm		
0,01	ns		
0,05	ns		
	Root mass		
LSD	Radifarm		
0,01	1,1542		
0,05	0,7619		

Statistical analysis of the data showed that the total garlic plant mass was under significant influence ($P \geq 0.01$) of treatment with biostimulant (Table 2). On plants treated with biostimulant highest total mass of 25.66 g had been recorded, while on control plant smallest total mass of 12,96 g.
The LSD test showed statistically significant difference ($P \geq 0.01$) in the garlic bulb mass between control plants and plants treated with biostimulant. The highest bulb mass was

recorded on treated plants 25,19 g, while the smallest bulb mass was recorded on control plants 10,54 g.
Treatment with biostimulant also showed a significant difference (P≥0.05) in number of cloves per bulb. The largest number of cloves was found in garlic plants treated with biostimulant 14, and the smallest in control garlic plants 7.

Table 2. Influence of biostimulant on total garlic plant mass, bulb mass and number of cloves per bulb of garlic

Garlic	Control	Treatment	Mean
Total garlic plant mass	**16,17**[b]	**20,56**[a]	**18,365**
Bulb mass	**15,27**[b]	**19,04**[a]	**17,155**
Number of cloves per bulb	**9,80**[b]	**12,74**[a]	**11,270**
	Total garlic plant mass		
LSD	Radifarm		
0,01	2,7054		
0,05	1,1729		
	Bulb mass		
LSD	Radifarm		
0,01	2.0896		
0,05	0,9059		
	Number of cloves per bulb		
LSD	Radifarm		
0,01	6.6886		
0,05	2.8998		

The result in this research showed positive affect of biostimulant on almost all investigated parameters of both wild rose and garlic. This might be due to the fact that biostimulants work by increasing mineral uptake and improving the nutrient use efficiency (Parađiković *et al*., 2013). Biostimulant used in this investigation consist of amino acid, vitamins, humic acid, glucosides, nucleotides and mineral nutrients. Stimulatory effects of humic acid on other plants were presented on lettuce (Young and Chen, 1997), tomato (Adani *et al*., 1998), garlic (Abdel-Razzak and E-Sharkawy, 2013), gerbera (Nikbakhta *et al*., 2008).
Similar results in recording greater number of cloves per bulb and achieving overall better morphological characteristic were presented by Shalaby and El-Ramady (2014) and (Abdel-Razzak and El-Sharkawy, 2013).
According to El-Nemr *et al*., (2012) positive effect on plant growth, fruit set and improvement of cucumber production can be achieved by application of biostimulators.

Conclusion

Positive effect of biostimulants on growth and development of almost all agricultural crops initiated increasing interest in using biostimulant in everyday crop production. This study confirms positive effects of biostimulant on all investigated parameters on both wild rose and garlic plants. Treated plants of wild rose had significantly greater root mass, while garlic plants had significantly greater total plant mass, bulb mass and number of cloves per bulb in comparison with control plants. Biostimulant application can be considered as good practice, especially for overcoming stress during the transplantation as well as improved plant growth and development after transplanting.

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