Effect of MF3 (peptidyl-prolyl cis/trans isomerase) protein from *Pseudomonas fluorescens* on *ex vitro* adaptation and post-adaptation of hardy kiwi (*Actinidia arguta* Planch. ex Miq.) plants

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Abstract. The effect of bacterial protein (peptidyl-prolyl cis/trans isomerase) in different concentrations on *ex vitro* adaptation and post-adaptation of *Actinidia arguta* Planch. ex Miq. plants of cultivar Geneva was investigated. The results indicating the growth-stimulating effect of MF3 protein were obtained, when applied at a concentration of 0.5 mg/mL, morphometric indices of above-ground plant development increased almost 2-fold compared to the control, and when used at a concentration of 0.1 mg/mL, there was a 1.8-fold increase in the root system compared to the control.

1 Introduction

Actinidias are woody perennial deciduous vines that have their origin in the forests of eastern Asia. Actinidia have a long history of collection and use in the wild throughout their habitat, both for food and as traditional medicine. The fruits are highly nutritious, containing ascorbic acid, B, PP, biologically active substances, macro and trace elements. They grow in the mountain forests of China, South Korea and neighbouring islands, with populations also found in Russia in the forests of the Far East, in Primorsky and Khabarovsk Territories, Amur and Sakhalin Regions [1-4].

The genus *Actinidia* Lindl. includes approximately 54 species, of which 44 are endemic to China. The most cultivated species, including those of industrial importance: heatloving specie – kiwifruit (*Actinidia chinensis* Planchon), cold hardness species have common name – hardy kiwis (*Actinidia kolomikta* Max., *Actinidia arguta* Planch. ex Miq., and *Actinidia polygama* (Siebold & Zuccarini) Max.)), and the less common *Actinidia giraldii* (*Actinidia giraldii* Diels. / *Actinidia arguta* var. giraldii (Diels.) Voroshilov), the latter two being protected species on the territory of the Russian Federation [5, 6].

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In temperate climate, the Actinidia arguta (*Actinidia arguta* Planch. ex Miq.) is most often cultivated in industrial plantations, as it and its varieties exhibit greater productivity than Actinidia kolomikta (*Actinidia kolomikta* Max.). Currently, industrial plantations are located in Asia, Europe, North and South America, and Australasia. The largest plantations in the world are located in China, covering an area of over 1,300 ha, in Europe, where they span approximately 400 ha, and in the USA, where they encompass 80 ha [7, 8].

To date, the largest plantation of Actinidia in Russia is an industrial site located in the central part of Russia, planted in NPC 'Agropischeprom', with an area of more than 1 ha.

The growing demand for actinidia fruits has led to an increased need for the production of high-quality planting material, which is typically obtained through traditional methods of vegetative propagation. The principal techniques employed for the production of planting material are green and woody cuttings, in addition to the method of off-shoot branching [9,10]. The seed method is not in demand, as it does not preserve the homogeneity of the genetic material of the mother plants. Furthermore, these propagation methods are inferior to the modern method, which produces a significantly greater quantity of planting material: clonal micropropagation (*in vitro*) [11-13]. Nevertheless, this method is associated with a number of challenges at various stages of the technology. In particular, at the stages of introduction into culture, adaptation and post-adaptation, a significant proportion of the plant material is lost [14].

It has been determined that leaves produced *in vitro* during the adaptation period may exhibit heightened sensitivity to environmental stressors. During the process of acclimation, leaves produced during subcultivation under in vitro conditions may exhibit intermediate characteristics between those of *in vitro* grown leaves and those of leaves grown in a greenhouse or in the field. Only the new leaves that have fully developed after removal from the *in vitro* culture resemble leaves that have been grown under conventional conditions. In contrast, roots formed *in vitro* culture display anatomical differences from roots grown *ex vitro*, which may result in a weakened absorptive capacity of adapted microplants [15].

A significant factor in the successful acclimation of plants to non-sterile environments is the development of their innate immunity, which enables them to withstand stressors such as photoinhibition, reduced humidity, and the presence of pathogenic microorganisms in the substrate [16]. However, evidence suggests that *ex vitro* microplants, even after acclimation, may exhibit growth cessation, which can considerably complicate the cultivation process [17].

There are numerous methods that can be employed to overcome such issues. The use of LEDs as a light source, the deployment of special adaptation greenhouses with automated humidity reduction systems, and the use of diverse preparations that induce plant immunity (i. e. elicitors) [18, 19] are some of the approaches that can be adopted.

Nevertheless, the use of such preparations comprising environmentally benign protein inducers of plant resistance to diseases may emerge as a viable alternative to synthetic preparations, given that under natural conditions such elicitors undergo decomposition to yield harmless natural amino acids [20, 21].

In recent decades, a number of novel elicitor proteins have been obtained and are currently being employed in agricultural applications. In particular, MF3 is a low molecular weight thermostable protein belonging to the peptidyl-prolyl-cis/trans-isomerase family of enzymes. The protein was initially obtained from the bacterium *Pseudomonas fluorescens* [22]. A number of laboratory and field studies have been conducted with the objective of confirming the protective effect of MF3 against phytoviruses, phytopathogenic fungi and bacteria on a range of crops, including tobacco, potato, wheat and others [23]. Additionally, in vegetation experiments on spring wheat seedlings, the capacity of the MF3 protein to stimulate growth and enhance plant condition in the presence of unfavourable and stressful environmental

factors was identified [24]. At present, there is no information in the literature on the issue how such a protein can influence *ex vitro* adaptation of microplants.

The objective of this research was to investigate the impact of bacterial protein MF3 (peptidyl-prolyl cis/trans isomerase) on the *ex vitro* adaptation and post-adaptation of hardy kiwi (*Actinidia arguta* Planch. ex Miq.) plants of the Geneva cultivar in greenhouse conditions.

2 Materials and Methods

The research was carried out in 2024 at the All-Russian Research Institute of Phytopathology, in Department of Molecular Biology and of the Laboratory for Recovery and Research on the Adaptive Potential of Crops and Plants.

The research objects were *ex vitro* microplants of *Actinidia arguta* Planch. ex Miq. obtained by clonal micropropagation and subcultured on a nutrient medium in accordance with the methodology described by Quorin-Lepoivre [25]. The recombinant MF3 protein was obtained in accordance with the methodology described by Erokhin et al. [21].

The microplants were transplanted into non-sterile conditions at 30 days after rooting. Microlants with 7-8 leaves and a root system consisting of 4-5 roots 4-8 cm long were used for adaptation. The sterile microplants were removed from the culture vessels, and the roots were thoroughly washed of the nutrient medium residues. The washed microplants were placed in pre-prepared experimental solutions of the protein. Plants were incubated in the prepared solutions of MF3 protein for 15 min.

Plant treatment options:

1. Plants treated with distilled water – control;
2. P1 – plants treated with MF3 protein at a concentration of 0.1 mg/ml;
3. P2 – plants treated with MF3 protein at a concentration of 0.5 mg/ml;
4. P3 – plants treated with MF3 protein at a concentration of 1 mg/ml.

Following the completion of the treatment phase, the microplants were relocated to mini-greenhouses with transparent lids and valves for ventilation, which were used for their subsequent adaptation. Each microplant was cultivated in a distinct compartment. The substrate employed was a soil-based mixture, designated 'Zemlya sadovaya', consisting of soil, perlite and vermiculite in a 4:1:1 ratio. At three weeks after transplantation, when the plants had begun to form new leaves, the mini-greenhouses were opened for ventilation. The adaptation phase was conducted in artificial climate chamber (Horaeus Votsch, Germany), with illumination levels of 1000-1500 lux, a 16/8 hour photoperiod, and a temperature of 20-22 ºC. The adaptation period was 40 days in duration, and then the plants were transferred to 250 ml pots and relocated to the greenhouse for pre-growth.

The microplants were completing growing in a greenhouse complex under natural light, with a temperature of 24°C during the day and 18°C at night, a photoperiod of 14/10 hours, and air humidity of 70%. Subsequently, the microplants were treated with the aforementioned protein solutions at the same concentrations as during the adaptation phase, once in every two weeks. The counts were conducted every 14 days. The parameters considered were the percentage of microplants establishment, the length and the number of shoots, the length and the number of roots, and the assimilation surface of the microplant.

The experiments were conducted in triplicate. The statistical processing was conducted using the Microsoft Excel 2016 and STATISTICA_10.0.1011 software, which validated the reliability of the obtained research results. The statistical significance of the mean value differences was evaluated using the t-test ($P < 0.05$). The data are presented as mean values and standard error of the mean ($M \pm SEM$).

3 Results and discussion

The results of the experiment demonstrated that treatment with an aqueous solution of MF3 protein had a significant impact on the *ex vitro* plants morphometric indices of actinidia arguta (*Actinidia arguta* Planch. ex Miq.) (Figure 1).

Fig. 1. Experimental *ex vitro* plants of actinidia arguta (*Actinidia arguta* Planch. ex Miq.), cultivar Geneva, treated at the tested concentrations, during of growing completion period.

On the 14th day of *ex vitro* of growing completion of actinidia arguta plants, the effect of MF3 protein treatment at a concentration of 0.5 mg/ml on the number of shoots was reliably revealed. It was 2.25±0.37 pcs. vs. 1.13±0.23 pcs. in control, and also the effect of MF3 on shoot length was revealed, which was 12.94±1.5 cm vs. 7.45±1.15 cm in control. Significant differences with the control in leaf surface area were observed in variants with MF3 protein at concentrations of 0.1 and 0.5 mg/ml – 106.36±11.11 cm^2 – 107.19±8.67 cm^2 vs. 67.83±10.57 cm^2 in control.

On the 28th day of *ex vitro* of growing completion of actinidia arguta plants, greater shoot length was significantly detected in the variant with MF3 protein at a concentration of 0.5 mg/ml and was 13.93±1.90 cm vs. 8.38±1.32 cm in the control, while reliable differences in the leaf surface area index were found in all experimental concentrations: 108.91±12.99 – 136.50±17.61 cm^2 vs. 77.68±12.46 cm^2 in control.

On the 42nd day of growing completion the advantage of the earlier isolated variants on the same morphometric indices was preserved, namely: the greater length of shoots was reliably revealed in the variant with MF3 protein at a concentration of 0.5 mg/ml and was 15.00±1.94 cm vs. 8.64±1.31 cm in the control, reliable differences on the leaf surface area index were revealed in all experimental concentration: 116.68±9.78 – 135.06±14.81 cm^2 vs. 77.33±12.80 cm^2 in control.

On the 56th day of observation of actinidia arguta seedlings in containers, a reliable difference with the control was revealed in all experiment variants in terms of shoot length and leaf surface area. The length of shoots was: 17.75±1.73 – 21.83±2.95 cm vs. 9.49±1.56 cm in control, and leaf surface area in the treatment was – 182.43±13.75 – 204.23±8.09 cm^2, vs. 101.28±17.29 cm^2 – in control, respectively (Table 1).

Table 1. The morphometric indices of *ex vitro* development of the aboveground system in actinidia arguta (*Actinidia arguta* Planch. ex Miq.). plants of the cultivar Geneva.

	Records of observations			
	14th day	**28th day**	**42th day**	**56th day**
Number of shoots, pcs.				
Control	1.13±0.23	1.38±0.32	1.38±0.32	1.88±0.40
P1	1.63±0.18	1.63±0.18	1.63±0.26	2.00±0.46
P2	**2.25±0.37***	2.50±0.46	3.00±0.93	4.75±1.45
P3	1.38±0.18	1.38±0.18	1.50±0.19	1.50±0.19
Length of shoots, cm				
Control	7.45±1.15	8.38±1.32	8.64±1.31	9.49±1.54
P1	9.50±0.80	10.34±0.95	12,08±0.94	**17.75±1.73***
P2	**12.94±1.50***	**13.93±1.90***	**15.00±1.94***	**21.43±2.95***
P3	10.63±1.60	10.93±1.78	13.68±2.04	**18.00±2.59***
Leaf surface area, cm^2				
Control	67.83±10.57	77.68±12.46	77.33±12.80	101.28±17.29
P1	**107.19±8.67***	**111.91±10.50***	**128.53±8.55***	**204.23±8.09***
P2	**106.36±11.11***	**136.50±17.61***	**135.06±14.81***	**192.26±24.99***
P3	89.88±12.11	**108.91±12.99***	**116.68±9.78***	**182.43±13.75***

*The results revealed that there were reliable, significant differences between the variants at the 5% significance level, as determined by the t-test (P<0.05).

Furthermore, the root system was analyzed both before and after of the growing completion period. The impact of treatment with MF3 protein on the root length at a concentration of 0.1 mg/ml was observed: 164.17±4.24 cm vs. 92.93±13.96 cm in the control group (Table 2, Figure 2).

Table 2. The morphometric indices of *ex vitro* development of the root system in actinidia arguta (*Actinidia arguta* Planch. ex Miq.). plants of the cultivar Geneva.

	Records of observations	
	Before of growing completion	**After of growing completion**
Number of roots, pcs.		
Control	8.00±0.71	10.67±1.20
P1	11.00±0.94	13.67±1.67
P2	12.00±1.41	12.00±1.00
P3	11.33±0.89	16.00±3.00
Length of roots, cm		
Control	76.50±9.64	92.93±13.96
P1	115.13±9.07	**164.17±4.24***
P2	119.27±9.76	147.63±4.35
P3	107.73±8.95	176.67±17.96

*The results revealed that there were reliable, significant differences between the variants at the 5% significance level, as determined by the t-test (P<0.05).

Fig. 2. The experimental *ex vitro* plants of actinidia arguta (*Actinidia arguta* Planch. ex Miq.), cultivar. Geneva, were measured at the time of root system observation : A – before post-adaptation; B – after post-adaptation (using MF3 protein at a concentration of 0.1 mg/ml)

The MF3 protein has been demonstrated to have a beneficial impact on the development of both underground and aboveground *ex vitro* systems of actinidia arguta (*Actinidia arguta* Planch. ex Miq.) plants in containers, particularly during the adaptation and post-adaptation stages. It can therefore be employed during these stages for better adaptation and post-adaptation of actinidia arguta plants, obtained by microclonal propagation.

4 Conclusions

On the 56th day of the of growing completion phase of the experimental *ex vitro* microplants, the advantage of using an aqueous solution of protein MF3 at a concentration of 0.5 mg/ml was revealed. The morphometric indices of the above-ground plant development increased almost twofold in comparison to the control. Additionally, when protein MF3 was used at a concentration of 0.1 mg/ml, the root system increased by 1.8 time in comparison to the control.

The results obtained confirm the growth-stimulating effect of MF3 protein, thereby opening up the possibility of its use at the adaptation and post-adaptation stage of actinidia plants obtained by clonal micropropagation. Further studies on the growth-stimulating effect of MF3 protein are recommended, potentially on the other crops.

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