MYCEPHYT APPLICATION IN THE ACCLIMATIZATION OF THE RASPBERRY *(RUBUS IDEAUS* L.) IN VITRO PLANTS

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Abstract. The mycephyt (10 ppm) treatment (30') of raspberry microplants positively influences their survival and rooting *ex vitro.* The effect depends on the way of treating, auxin medium content and the length of the preliminary stage on the medium, containing 1BA (0, 5 mg/1) before microshoots transfer to the non sterile conditions.

Key words: acclimatization, raspberry, mycephyt, micropropagation, hormones.

Introduction

The acclimatization of raspberry plantlets in the non sterile conditions is one of the labor intensive and important stages in micropropagation of the garden plants: the regenerant losses may be very significant [2, 3].

The results of root induction of softwood cuttings after mycephyt treatment allowed us to suppose their positive influence on the successful adaptation of the plantlets, rooted in vitro and the direct rooting of berry microshoots under ex vitro conditions. The direct rooting can be taken as the connecting link between in vitro and in vivo propagation to reduce the growth time and the production cost. The high sensitivity of the microcuttings or rooted microplants to stresses makes it necessary to seek out ways of their rapid stabilization and further vigorous growth in a greenhouse.

Mycephytes have a distinguished rhizogenic activity (10-100 ppm) and provide high resistance under unsuccessful growth conditions [1]. Mycephytes, received on the endotrophic fungous basis, promote immune properties and positively effect the host plant growth due to the high hormone activity, optimal mineral nutrition, the intensive assimilation processes and active metabolism [4]. Mycephyte is the sterile lyophilized balanced range of biological active substances (carbohydrates, amino acids, fatty acids, hormones, such as cytokinins, gibberellins, auxins), effective in a low concentrations. It's high physiological activity goes with a low toxity. The effect of biostimulation reveals itself especially under unsuccessful growth conditions: poor soils, water deficit and environmental pollution.

Materials and methods

Researches were conducted in 2008-2010 at the horticultural laboratory in Russian state agrarian university (RSAU) — Moscow agricultural academy called after Timiriazev. The objects of the investigation are the cultivar of red raspberry (Geracl) and the hybrid between blackberry and raspberry (Tayberry).

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Aseptic culture was established on Murashige and Skoog medium (MS), containing the main macro-, microelements supplemented with (mg/1): B_1, B_6 and PP (0.5), mesoynositol (100), glicine (2), 6-benzilamino purine (BAP) (0, 7), indole-3 butyric acid (IBA) (0.1), sucrose (30000) and agar (7000). PH value — 5.6. The cultures were incubated at a constant temperature of 22° C and light photoperiod — 16 h.

At the stage of multiplication the medium was modified by increasing of FeEDTA 1/5 times (BAP 1.5 mg/1). At the rooting stage macrosalt's content was reduced twice (ІІІА 1 mg/1). In further trials microshoots were kept on the medium with ІІІА (0.5 mg/1) or without hormones during 5, 10, 15, 20, 25, 45 days before their direct rooting in a greenhouse under « mist» system in the plastic cells (d 10 cm), containing substrate (peat: perlyte = 1:1).

Before the microplants transfer they were treated with the mycephyt water solutions (1, 10 ppm) during 0, 5; 1 and 5 h.

Resuilts and discussion

After 6 weeks of acclimatization the percentage of the plants acclaimed, their height, number and length of roots, the development of leaf area were appreciated (table 1).

The distinguished differences with control in adaptation rate and microplant development of the hardly propagated remontant cultivar *GeracI* were revealed in a variant with a short (30 ') mycephyt treatment (10 ppm): number of acclimated plantlets accounted for 88.7 against 35.7% in the control. All plants were well formed and had the highest data of shoot length and the total leaf area. The treatment during 2-5 hours led to the adaptation decrease. The use of the lower concentration (1 ppm) was inexpedient.

Table 1

The mycephyt influence on the acclimatization of the raspberry microplants under non sterile conditions

Variants	Concentration, ppm	Exposure, h	Adaptation, %	Share of vigorous plants, %	Shoot length, cm	Number of new leaves, items	Total leaf area, cm^2
c. Geracl							
Control (without treating)	—		35,7	37,5	2,0	2,4	17,4
Mycephyt	10	0,5	88,7	100	3,4	3,8	37,5
		2	68,6	100	2,8	3,2	24,5
		5	60,2	100	2,5	2,7	20,3
	1	0,5	61,4	52,4	2,4	2,6	26,5
		2	46,8	34,1	2,1	2,8	24,2
		5	34,2	31,2	2,3	1,9	18,8
LSD_{05}			16,2	12,8	0,6	0,7	3,2
c. Tayberry							
Control (without treating)	—		70,2	28,5	1,1	2,1	16,1
Mycephyt	10	0,5	97,2	49,2	2,0	2,7	35,2
		2	87,2	28,7	1,9	1,8	20,2
		5	80,7	18,3	1,4	1,5	18,4
	1	0,5	85,8	23,8	1,6	2,2	20,5
		2	85,1	22,6	1,5	1,9	19,3
		5	80,3	24,8	1,1	2,1	15,8
LSD_{05}			12,3	10,4	0,5	0,5	3,9

In the trials with moor simply propagated cultivar *Tayberry* the result was just the same: the short mycephyt treatment provided the best survival of regenerants. But in spite the high in vitro plants viability their initial growth was less vigorous and mycephyt application provided a low amount of high quality plants adapted.

In further experiments microshoots were rooted in the greenhouse directly in the substrate. After 8 weeks plantlets were evaluated for rooting and growth capacity. The microshoot mycephyt treatment (10 ppm) essentially effected the root formation. The highest rooting percentage obtained was 100 against 86 % in a control. The positive correlation between this indicator and succeeding growth was revealed. Thus the root forming (c. *Yellow Giant)* start was 10 days earlier and the rooting rate increased by 100 against 65% in the variant without treatment.

It is well known, that the adaptation depends on the last stage of micropropagation, the hormone and mineral content of the medium promoting axillary's shoot proliferation.

Several methods for microshoot rooting of remontant raspberry *Geracl* have been tried according to the length of the last stage on the medium with IIIA (0.5 mg/1) and further mycephyt application (10 ppm, 30'). The trial shoots were subcultured on the auxin medium during different expositions from 5 to 45 days before they were transferred to the substrate.

Part of the already rooted microplants was precultured with hormones or on hormone free medium for 45 days before their transfer ex vitro (additional control).

The values of rooting and development of raspberry regenerants are presented in table 2.

T a b l e 2

The effect of hormone content of the medium on the stage before transfer, its length and the mycephyt treatment of raspberry microshoots on their rooting and acclimatization ex vitro (c. Tayberry)

Variants	Exposition on the IBA medium, days	Mycephyt treatment	Adap-tation, %	Shoot length, cm	Number of leaves, items	Leaf area, cm^2	Total leaf area, cm^2	Share of vigorous plantlets, %
Microshoots								
Control (6-BAP)		–	86,0	3,2	4,5	7,5	33,8	84,4
		+	100	3,5	4,5	8,8	39,6	57,1
IBA (0.5 mg/l)	5	–	100	3,1	6,9	7,5	51,8	100
		+	100	2,7	6,0	7,4	45,1	100 *
	10	–	100	3,2	6,4	7,5	48,2	100
		+	100	3,9	4,0	13,5	54,3	86,1*
	15	–	100	4,7	5,5	12,1	66,4	86,3*
		+	100	5,9	7,2	12,4	84,3	100**
	20	–	100	6,7	5,6	7,5	50,1	100**
		+	100	7,3	6,7	15,8	88,5	100**
	25	–	100	4,8	5,8	12,1	69,6	100*
		+	100	4,6	4,5	16,1	72,4	100**
Microplants rooted								
IBA (0.5 mg/l)	45	–	100	6,7	4,4	20,4	88,1	100**

*** well formed root system; ** Vigorous roots, which fill up the whole volume of plastic cells.**
LSD ^for isolated differences (indicator «shoot length») — 1,1
LSD и for isolated differences (indicator «total leaf area») — 9,8

The best rooting ex vitro (the increase by 100%) and the high quality of rooted *Tayberry* plants were obtained after short (15-25 days) cultivation on the medium with IBA (0.5 mg/1). The effect of mycephyt treatment (30') rose significantly: 1, 4-2, 3 times increased the plantlet height; 1, 3-2, 3 times — total leaf area. All plantlets adapted had a developed root system: share of vigorous regenerants — 100 against 57% in a control; roots filled up the whole volume of plastic cells. In these variants the results of rooting and acclimatization were better, than after transfer of microplants rooted in vitro.

Thus we can shorten the multiplication cycle, optimize the acclimatization, and stimulate the initial active growth and development. This gives the opportunity to receive a high quality planting stock of raspberry in a shorter time.

Rooting the hardly propagated raspberry cultivar *GeracI* ex vitro the preliminary stage on the medium with IBA must be shorter (10-20 days) otherwise the efficiency of the mycephyt application will sharply decrease. In spite of the successful rooting ex vitro in some variants (IBA 15 days and the following mycephyt treatment) we must point out a significant difference in acclimatization and further development of shoots rooted in substrate and plantlets, rooted in the sterile culture: the last one grew better (table 3).

When plantlets rooted in vitro were cultivated for a long period (45 days) on the hormone free medium the mycephyt treatment was not necessary. The same expositions of already rooted microplants on the medium with IBA slowed down both rooting and

Table 3

The effect of hormone content of the medium on the stage before transfer, its length and the mycephyt treatment of raspberry microshoots on their rooting and acclimatization ex vitro (c. GeracI)

Variants	Exposition on the IBA medium, days	Mycephyt treatment	Adap-tation, %	Shoot length, cm	Number of leaves, items	Leaf area, cm^2	Total leaf area, cm^2	Share of vigorous plantlets, %
Microshoots								
Control (6-BAP)		–	57,1	2,2	3,0	6,8	20,4	50,2
		+	86,9	1,2	2,8	8,1	22,7	34,1
IBA (0.5 mg/l)	5	–	74,2	2,1	2,1	8,4	8,9	28,4
		+	86,4	1,9	3,8	4,0	15,2	33,6
	10	–	71,6	1,9	2,9	4,1	11,6	0
		+	86,1	2,8	7,3	2,3	16,8	50,4*
	15	–	49,2	3,2	6,1	8,2	49,2	84,3**
		+	100	3,4	11,7	9,4	109,9	100**
	20	–	100	2,4	3,6	7,5	73,5	42,9*
		+	100	3,1	9,8	8,8	31,7	71,4*
	25	–	71,4	1,6	3,8	1,0	3,8	0
		+	100	2,1	3,5	2,0	7,2	0
Microplants rooted								
IBA (0.5 mg/l)	45	–	57,2	3,0	7,1	6,3	44,1	85,7
		+	94,3	4,1	8,2	9,4	77,1	100*
Hormone free medium	45	–	100	5,6	8,7	16,1	139,2	100*
		+	100	5,7	5,9	20,3	119,8	100**

*** well formed root system; ** — vigorous roots, which fill up the whole volume of plastic cells.**
LSD $_{05}$ for isolated differences (indicator «shoot length») – 1,1
LSD o,for isolated differences (indicator «total leaf area») — 9,8

shoot development up to reverse connection type. In this case the mycephyt application increased the survival of regenerants, provided a high percentage of adapted plants and good development of root system and leaf surface.

In this way the micephyt application had a positive effect on the efficiency of the multiplication of raspberry cultivars and acclimatization of regenerants under non sterile conditions, using two ways of rooting, in vitro and ex vitro. The method is suitable for rapid micropropagation of raspberry plants for the production of healthy stock plantations.

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Аннотация. Кратковременная (30') обработка микропобегов малины мицефитом (10 ррш) оказывает положительное влияние на их приживаемость и укоренение *ex vitro*. Эффект зависит от способа обработки и длительности предварительного этапа на среде с ИМК (0,5 мг/л), предшествующего пересадке микропобегов в нестерильные условия.