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BIOLOGICAL CONTROL OF GROWN GALL IN HORTICULTURE

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Abstract

Crown gall is a widespread disease of cultivated culture all over the world. The bacterial pathogen Agrobacterium tumefacience colonize the xylem vessels of the plants.The tumors form in stems and roots of fruits culture. Large tumoursmay death of new plants in the nursery and in the plantation. Tumouregenesis causing root decay may be factor involved in the complex <replant disease> syndrome. Development of measures to controle crown-gall is carried out in direction for obtaining tumorless plants at the expens of preventive treatment of woundings so as to deteriorate the interaction of pathogen with the cell of host plant. Utilization the strain of soil inhibiting Pseudomonas fluorescens CR-330 D which synthesize the substances (bacteriocins) with preventing activity to nopaline and octopine strains of pathogenic agrobacteria. Liquid concentrate suspension of this bacteria - biological preparat < Paurin> -were applicated for preplanting treatment of rooted saplings. Using <Paurin> to treat apple MM-106 wilding prior to planting into fruit nursery allowed to decreases the outcome of apple saplings affected by crown gall down to 2 – 2.5 %. He is capable to compete with soil microflora and get reliably fixed in rhisosphere without phytotoxicity, which is rather advantageous for using biological control against repeated inoculation with crown gall pathogen.

Keywords: grown gall, bacterial strain, biological control.

INTRODUCTION

Microbial diseases affect numerous plant crops, resulting in huge loss and decrease in quality and safety of agricultural products. Crop disease control relies mainly on chemical pesticides that are currently subject to strong restrictions and regulatory requirements.
Microorganisms continue to play a highly significant role in the discovery of antiinfectives by producing a wide range of antimicrobials. Biocontrol of plant pathogens through bacterization technique has gained momentum, and *Pseudomonas*, one of the most diverse Gram-negative bacterial genera, had been selected for the study. *Pseudomonas fluorescens*, a plant growth-promoting rhizobacteria (PGPR) widely distributed in soil, has the ability to colonize the rhizosphere of host plants and produce a wide range of compounds inhibitory to a number of serious plant pathogens
Grown gall is diseases that are large number of plants-hostes. The causative agent of disease is gram-negative bacteria *Agrobacterium tumefaciens* (Smith and Towns) – the heterogene, presented in the nature by various strains with specific properties depending on a plant. In fruit nurseries of Moldova the tumors of a grown gall develop on roots of vegetative multiple stocks of an apple-tree, seed stocks of stone cultures and in places of graft (Figure 1).

Figure 1. Young apple plant with symptoms of grown gall

Their growth breaks of nutrients intended for plants. The quantity of saplings of an apple-tree on a stock of M-4 reaches 65.4%, on M- 9 –

24.3%, on MM-106 – 14.7%. At an grafting on seed stocks defeat of saplings of a pear makes 42.4%, a peach on almonds-3.2 %, an apricot on zherdele-5.8%, plums on a cherry plum-5 %, sweet cherry-5%. For this reason annual saplings in nursery often don't conform to the standard and after plant to a garden trees lag behind in growth and often perish, without having reached fructification. Many yellow leaves, troncs with less volume are visually observed. The attacks of plants in gardens with weak and average damaged roots make to 18% from total number of plants on one hectare. In some gardens of plum and a peach about 94% of trees are struck. Tumors are result of genetic interaction of agent parasite in plants cell, the consequence control of disease can't be solved by the standard chemical methods. Problem of science is searching of alternative systems of the prevention and phytopathogens a grown gall at the expense of application of ecologically safe biological preparations of a microbiological origin. The particular interest in this sense is representing the bacteria pick from rizosphere of plants.

Team of researchers from Institute of molecular genetics of the Russian Academy of Sciences on the basis of studying of numerous isolates of bacteria, allocated around a radical zone of plants, selected the strain of *Pseudomonas fluorescens* CR-330D producing the bacteriocins - the low-molecular substances of not proteinaceous nature suppressing activity of *Agrobacterium tumefaciens* (Khmel et al.,1995).

MATERIALS AND METHODS

Two-daily suspension of living bacterial cells *of Pseudomonas fluorescens* CR-330D, with the dilution 10^7 CFU/mL was applied to spraying plants before a sowing and landing material. Bacterial suspension of a strain antagonist of the causative agent of grown gall use for treatment the stouns of cherry, plum, peach, and also the layers of vegetative increased scrops of an apple rootstocks before plant in nursery, sprayed the places of woundings on mother bushes of rootstocks. Before planting of fruit trees in the garden the suspension of *Ps. fluorescens* used for bacterization of root system of a landing material year-old sapling fruit trees and added them with irrigation water. The biological preparation on the basis of strain *Pseudomonas fluorescens CR-330D*-"PAURIN" is registered by the State center for certification of phytosanitary production in the Republic of Moldova.

RESULTS AND DISCUSSIONS

In time of vegetative multiplication the rooting stem of rootstocks of apple MM 106, M 26, and the bacterization the roots of scions before landing of the first field of nursery was carried out. In time of dig ap the saplings from nursery the essential decrease galled roots on landing material of an apple-tree after treatment by PAURIN in relation to control was observed. The received results are presented in the Table 1.

Table 1. Influence of prelanding treatment of rootstocks with "PAURIN" on gall inhibition of roots the saplings in nursery (2011-2012 years)

Breeds	Rootstook	Variants	Quantity of plants	Galle-daplings piece	% of disease	Biological effect %
APPLE	MM-106	Control	1500	246	16,4	-
	M-26	Control	1420	112	7,9	-
	MM-106	Paurin	1450	37	2,6	84,1
	M - 26	Paurin	1210	16	1,3	83,5
Sweet cherry	Bitter cherry	Control	1620	68	4,2	-
		Paurin	1540	12	0,8	80,9
Plam	Wild plam	Control	2300	73	3,2	-
		Paurin	2300	9	0,4	87,5

Treatment the layers of vegetative stock of an apple-tree (M106 and M26) before landing in the first field of nursery by biopreparat "PAURIN" suspension promoted down to infection by grown gall to 2.6% -1.3% at 16.4 – 7.9% in control where landing of layers was made without biological product. Thus, biological efficiency of a biological product of PAURIN if compared to control against grown gall in time of raise saplings of an apple in nursery made 84.1% and 83.5% depending on a stock clone. After bacterization of stones of sweet cherry and a wild plum by preparation PAURIN suspension the yeield of healthy saplings of these breeds increased by 5-8 times in comparison with control. Biological efficiency of biofungicide in react against a grown gall at cultivation of saplings of sweet cherry and a cherry plum made 80.9% and 87.5%. Before landing of gardens with rooting apple saplings take bacterisation of root system

of saplings by PAURIN biological product on the total area of 86 hectares. Submitted data testify to considerable decrease in galled roots of apple trees in comparison with control (Table 2).

Table 2. Influence of prelanding treatment the roots of apple saplings by "PAURIN"on gall inhibition of roots the saplings in garden (2011-2012 years)

Breeds	Rootstook	Variants	Quantity of plants	Galled (piece)	% of disease	Biological effect %
Apple	MM-106	Control Control	1000	143	14.3	-
	M-26		1020	97	9.7	-
	MM-106	Paurin	1000	24	2.4	83.2
	M - 26	Paurin	1000	12	1.2	87.6

On materials of the table show that in control apple saplings on a rootstock of MM106 are infected with grown gall more (14.3%), than on M26–9.7% ,while after PAURIN treatment the number of plants with tumors decreased by 6-9 times and made 2.4%-1.2% at 16.4%-9% in control. Biological efficiency of biofungicide Paurin to controle against a grown gall when landing young saplings of an apple made 83.2% and 87.6%.

Pseudomonas fluorescens is plant growth promoting rhizobacteria (PGRB) inhabit the soil around the root surface and involved in promoting plant growth and development via production and secretion of varios regulatory in the vicinity of rhizosphere. PGRB facilitate the plant growth directly by either assisting resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant by controlling or inhibiting them and development in the forms of biocontrol agents (Munees and Kibret, 2014). This strain *Pseudomonas fluorescens* 330-D may be utilise as bio-inoculants to promote plant grow, improve the development of root system of plants. The use the suspension liquid of *Pseudomonas fluorescens* with titre 10^7 ucf/mL before the planting the samplings of vine in soil (50ml/kg soil), their application in time vegetation period by foliar pulverization has been studied (Table 3).

The dates of table show significantly increased plant dry weight, radicle and shoot length, shoot weight of own root samplings of vine.

Table 3. Influence the treatment of bacterial suspension *Ps. fluorescens* (titre10^7ufc/ml) on development the own root samplings of vine sort "Codrinskii"

Variants	Weight of roots (g) 1 plant (M±m)	Length (cm) of roots increase1 plant (M±m)	Length (cm) of shoot increase 1 plant	% to control
1	2	3	4	5
Control	7.58± 2.78	16.09± 2.32	41.04± 3,71	100
Ps. fluoresc. bring in soil	5.10± 0.63	16.30± 1,37	30.83± 3.18	75.12
Ps. fluorescens pulverisation of leavs	9.04± 2.26	23.80± 3.92	51.91± 8.85	126.5
Ps. fluorescens in soil + pulverization of leavs	10.68±0, 89	28.75±2.64	48.34± 4.19	117.7

It may be explained by properties of metabolits secreted by bacterial cells: phytohormone auxin (IAA/indol-3-acetic acid). This substance interferes with the many plant development processes because the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria. IAA increases the rate of xylem and root development, controls processes of vegetative growth, initiated lateral and adventitious root formation, affects photosynthesis, pigment formation, increases root surface area. Interaction of *Ps. fluorescens* with the plant root or shoot can result in plant resistance to phytopathogenic bacteria. This phenomenon is called induced systemic resistance (Lugtenberg and Kamilova, 2009).

Pseudomonas fluorescens have been reported to produce antibiotic compounds like phenazines, pyrrole derivatives, indole derivatives, 2.4-diacetylphloroglucinol etc. (Ahil Sajeli Begum et al., 2014).

When laying a new garden apple trees (varieties Champion, Florina, Idared, Jonagold, grafted on rootstock M26), the roots of seedlings were treated with biologic PAURIN to prevent bacterial infection of plants root cancer (*Agrobacterium tumefaciens*).

To improve rooting and growth of young trees start to the working solution (5 L of the bacterial suspension per 500 liters of water) was added 0.02% trace elements. Exposure soaking the roots of seedlings was 30 minutes. After planting, watering the plants was carried out with an aqueous solution of a biological

product rate of 0,5 L Paurin one tone of water. Survival of plants in the garden was 100%. Counts and parametric measurements were performed in the middle of summer and the end of the growing season by 30 experienced trees. In early August in the embodiments of application of the bacterial strain showed a significant increase in the length of the young compared to the growth control. Area of the leaf treated plants were larger and differed by more intense color.

At the end of the growing season on apple varieties Florina, the total growth of shoots treated before planting trees ranged from 7.4 to 9.2 m on the majority of the trees in the garden evolved 16-18 shoots length reaches 40-76 cm. Seedling stem diameter spray averaged 3.4 cm, which is 47.8% more than in the controls (2,3 cm). In the control, all indicators are significantly inferior variant of the experiment is presented in Table 4.

Table 4. Growth-stimulating effect of a biological product Paurin in combination with trace elements at preplant roots apple varietie Florina

Parameters	Version control	Experimental variant
The average area of the leaf, cm^2	76,4	92,1
Average number of shoots, pieces	12,5	16,7
The average length of the shoot, (cm)	29,8	43,2
The average diameter of the trunk, (cm)	2,3	3,4
The average length of the total growth, (cm)	372,4	726,2

CONCLUSIONS

Preplanting treatment of vegetative stocks of an apple-tree and seeds of stoun-fruit cultures with "PAURIN" (titre 10^7 cfu/mL) reduced level of a disease of fruit plants in comparison with control (biological efficiency of 80.9% to 87.5%) depending on breed.

Biological product PAURIN application in complex with micronutrients at planting apple seedlings strongly stimulated plant growth.

Bacterisation by biofungicide "PAURIN" the roots of saplings of an apple before landing in a garden reduced number of no galled plants to 2.4-1.2% in comparison with control of 14.3-9.7%. The received results testify that the biological product PAURIN can be applied successfully to prelanding bacte-rization of root system vegetative sets of apple rootstocks and seedlings of stoun fruit crops in nursery and saplings at a laying of young gardens to control against a grown gall.

Use of these properties, along with primary development of the microorganism on surfaces of stones, roots, shanks, a layer, tronc plants and in a rizosfer creates a physical and biochemical obstacle to development of pathogenic strains of the agent of a root cancer, improve the development of root system and shoots of plants.

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