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Impact of Microorganisms Strains on Diversity of Epiphytes of Tomato Seeds

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Results obtained in our study suggest that introduction of microorganism strains into rhizosphere of plants and epiphytic microflora of tomato seeds causes changes in composition of microbial environment of seed surface resulting in increase in diversity of microorganisms. Inoculation of tomato seeds by strains – bio-agents of bio-fertilizers, namely *Azotobacter vinelandii* 10702 – Azotobacterin, *Enterobacter nimipressuralis* 32-3 – Phosphoenterin, *Paenibacillus polymyxa* P – Biopolicide and *Rhizobium radiobacter* 10, which is a basis of Agrophyl, causes changes in composition of microbial diversity of seed surface, which is observed during the entire period of storage. Microorganisms are able to stay preserved on the surface of tomato seeds, with the longest period of time observed for sporous strain *Paenibacillus polymyxa* P. High rate of reproduction of *Enterobacter nimipressuralis* 32-3 strain at the beginning of the seed storage allows the occupation of the niche for long period of time. Inoculation of seeds with non-spore-forming *Rhizobium radiobacter* 10 and *Azotobacter vinelandii* 10702 strains causes similar effect on epiphytes formation, promotes increase in diversity of strains even after 78 months of storage, according to the highest Shennon indices of 1.739 and 1.749, respectively, and the lowest indices of domination - 0.2777 and 0.3614, compared to 0.5297 and 0.7829 in control. We also observed an increase in vigour of tomato seeds obtained from inoculated plants as a result of the influence of strains.

Key words*:* microorganisms, strains, tomato seeds, germination, epiphytes, diversity index.

Plants represent a complex ecological system for a significant number of microorganism strains, which have occupied particular niches for their existence as a result of evolution. It is known, that the microorganisms are found on the surface of seeds or growing plants and some of them are unable to reproduce and, therefore, disappear, whereas epiphytes are able to survive and develop in this ecological niche[3, 9, 11].

Structure of diversity of microorganisms associated with plants as well as their spatial differentiation in phytosphere are determined by numerous factors, such as climatic zone, type of soil, humidity, season, weather conditions, age of a plant and plant metabolites[4, 5, 17]. Treatment of seeds with bio- fertilizers which are based on strains of economically-useful microorganisms reduces the amount of other microorganisms on the surface.

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Introduction of the strains into rhizosphere of plants causes changes in quantitative ratio of particular ecological and trophical groups of microorganisms in microbial diversity of rhizosphere[2, 10, 13]. For example, inoculation of bio-fertilizers both separately and in mixture results in reduction in the number of micromycetes on surface of winter wheat seeds, as well as in the amount of micromycetes, actinomycetes and oligotrophes in the rhizosphere [13]. It was observed that in the conditions of mass distribution of toxic and phytopathogenic fungi at particular winter wheat locations, the seeds obtained from inoculated plants were healthy.

The aim of our work was to study the impact of strains, that are bio-agents of bio-fertilizers, on diversity of microbial epiphytes of tomato seeds and their quality.

MATERIALS AND METHODS

The seeds of tomato variety 'Chance' used in the study were obtained from plants inoculated by strains with different dominant features, namely nitrogen-fixation - *Azotobacter vinelandii* 10702, phosphate-mobilization *Enterobacter nimipressuralis* 32-3, antagonism to phytopathogenes – *Paenibacillus polymyxa* P. These strains are bio-agents of the following bio-fertilizers: Azotobacterin, Phosphoenterin and Biopolicide, respectively. *Rhizobium radiobacter* 10, which is a basis of Agrophyl, was used as a referent strain.

Seeds were treated with appropriate strains and kept for long-term storage of 78 months duration. The seeds obtained from inoculated plants, but not treated before storage, were used as a control. Then the seeds were analysed for their sowing quality.

The determination of the quantitative and qualitative composition of epiphytic microorganisms was carried out by surface sowing on agar media selective for particular strains[16]. Sowing quality of the seeds was determined by standard methods[12]. DNA of microorganisms was isolated from the seeds according to generally accepted protocol[1].

RESULTS

Results obtained from our analysis revealed that introduction of microorganism strains into rhizosphere of plants and epiphytic microflora of tomato seeds causes changes in composition of microbiological diversity of seeds. We studied changes of bacterial quantity on surface of tomato seeds obtained from inoculated by different bacterial strains plants (Figure 1). After 30 months of seed storage, variant with treatment by *P. polymyxa P* strain had the most significant number of bacteria on the seed surface, precisely $34E10^3$ CFU/g.

The analysis of fungi composition in epiphytic flora of tomato seeds showed a rapid decline in their quantity.

Microorganisms are able to stay on the surface of tomato seeds, in particular, the sporous strain *P. polymyxa* P, which is active agent of Biopolicide, can be preserved for the longest period of time. Its presence on the surface of tomato seeds

Table 1. Influence of microbial strains on diversity indices of epiphytes of tomato seeds after 78 months of eed storage duration

	Control	*P.polymyxa* P	*E.nimipres-suralis* 32-3	*R.radio-bacter* 10	*A. vinelandii* 10702
Dominance D	0.7829	0.5846	0.5006	0.2777	0.3614
Shannon H	0.5297	1.091	1.295	1.739	1.749
Simpson 1-D	0.2171	0.4154	0.4994	0.7223	0.6386
Evenness e^H/S	0.2426	0.1354	0.2283	0.3349	0.1854
Menhinick	0.4308	1.106	1.281	1.34	1.537
Margalef	1.076	3.511	2.97	3.149	4.993
Equitability J	0.2722	0.353	0.4672	0.6138	0.5093
Fisher alpha	1.32	5.023	4.468	4.799	7.802
Berger-Parker	0.8826	0.7576	0.6987	0.4658	0.5823

was observed after 56 months of the storage duration and after 78 months of the storage the highest index of strain domination was detected, as high as 0.5846.

InoAulation of tomato seeds by microorganism strains causes changes in rhizosphere of plants, as well as, in epiphytic microflora composition of microbial environment of seeds surface and resulting in increase in diversity of microorganisms. Thus, in cases when microbial fertilizers were applied the Shennon index was higher, particularly, in seeds obtained from inoculated plants by 2.8 – 3.4 times and with repeated inoculation by 2.1 – 3.3 times, suggesting the higher diversity in groups of epyphites (Table 1).

Increase of Menhinick's index by 2.3 – 3.6 times and Margalef index by 2.7 – 3.2 times was observed in species composition of epiphytes of inoculated seeds.

It is well known, that, the higher the domination index of the strains the lower the diversity of strains. During the analysis of strain composition of epiphytes of seeds from inoculated plants after their storage, it was observed that the domination index was decreased by 1.3 to 2.8 times in the group with extra inoculation before storage of the seeds with duration of 78 months in comparison with the control.

According to Table 2, after 42 months of storage duration the seeds obtained from inoculated plants displayed an increase in indices with regard to control: Menhinick's index by 1.4 to 1.9 times and Margalef index by 1.9 to 2.5 times.

The decrease in domination index by 2.0 to 3.7 times with regard to control was observed after 42 months of storage of seeds obtained from inoculated plants suggesting increase of diversity of epiphytes of seeds, caused by inoculation of bacteria with different dominant functional activity.

The analysis of general similarity of communities according to diversity indices has showed high level of similarity of microbial communities of the control regardless of storage duration (Figure 2).

Table 2. Influence of strains on diversity indices of epiphytes of tomato seeds after 42 months of storage duration

	Control	*A. vinelandii* 10702	*P.polymyxa* P	*E.nimipressuralis* 32-3
Dominance D	0.7709	0.3142	0.2095	0.3819
Shannon H	0.5761	1.628	1.967	1.462
Simpson 1-D	0.2291	0.6858	0.7905	0.6181
Evenness e^H/S	0.2224	0.3637	0.3575	0.2397
Menhinick	0.6154	1.171	1.28	0.89
Margalef	1.365	2.619	3.456	2.827
Equitability J	0.2771	0.6167	0.6566	0.5058
Fisher alpha	1.746	3.843	5.158	3.85
Berger-Parker	0.8757	0.5175	0.3689	0.5892

Table 3. Sowing quality of the tomato seeds obtained from inoculated plants extra treated before storage.

Variant of experiment	Duration of storage is 78 months			
	Vigour		Germination	
	%	% to control	%	% to control
Control	23.3	100	50.7	100
Rhizobium radiobacter 10 Agrophyl	50.3	215.8	75.3	148.5
A. vinelandii 10702 Azotobacterin	39.0	167.4	76.7	151.3
E. nimipressuralis 32-3 Phosphoenterin	47.3	203.0	81.0	159.8
P. polymyxa P Biopolicide	33.0	141.6	79.3	156.4
The LSD_{05}	12.53		7.95	

Also high level of similarity was detected in epiphytes of seeds inoculated by strains of *E. nimipressuralis* 32-3 with high rate of reproduction and *P. polymyxa* P with high competitiveness with respect to the epiphytes.

The positive impact of the strains, bio-agents of bio-fertilizers, is confirmed by the increase in sowing quality of seeds after 78 months of storage (Table 3). In fact, an increase in the vigour of tomato seeds obtained from inoculated plants, which were extra treated before storage, was shown to be by 41.6–115.8 % with regard to the control. Germination of the seeds was also increased after inoculation by 48.5 – 59.8 % in respect to the control.

DISCUSSION

We have previously reported that strains of microorganisms, which are bio-agents of bio-fertilizers have different ability to colonize tomato plants and have different levels of associative interaction with plants[6, 14, 15]. Strains can develop in the rhizosphere and cause an increase in roots mass of seedling and plant productivity.

In cases when seeds from inoculated plants were treated by bio-fertilizers, the amount of epiphytes after 6 months of storage was higher by 1.5 – 10 times, and micromycetes – lower by 2 – 4 times compared to control without any treatment. The best conditions for preservation of epiphytes are on seeds obtained from inoculated plants: their amount after 30 months of storage increased by 2 – 7 times compared to inoculation of seeds from non-inoculated plants[7, 8]. This process should be considered as positive due to the improvement of sowing quality of the seeds.

Thus, results of our study suggest that inoculation of tomato seeds by strains – bio-agents of bio-fertilizers causes changes in composition of microbial diversity of seed surface, which is observed during the entire period of the storage. High rate of reproduction of *E. nimipressuralis* 32-3 strain at the beginning of the seed storage allows the occupation of the niche for a long period of time. Inoculation of seeds with non-spore-forming *R. radiobacter* 10 and *A. vinelandii* 10702 strains causes similar effect on the formation of epiphytes, promotes increase in diversity of strains even after 78 months of storage, according to the highest Shennon index of 1.739 and 1.749, respectively, and the lowest indices of domination, as law as 0.2777 and 0.3614 compared to 0.5297

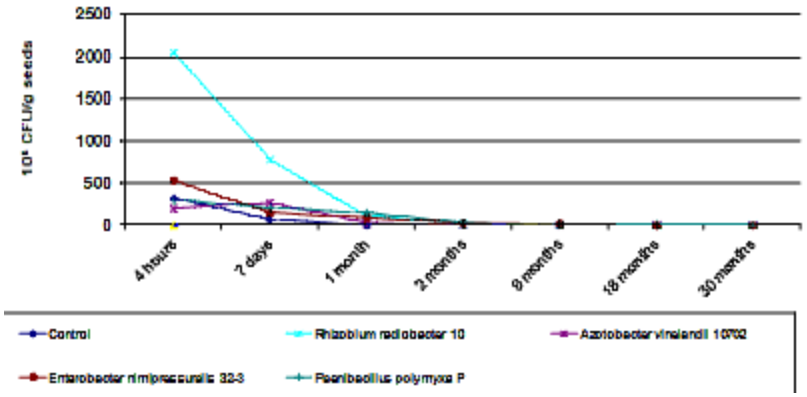

Fig. 1. Population dynamics of bacterial quantity at surface of tomato seeds obtained from the inoculated plants.

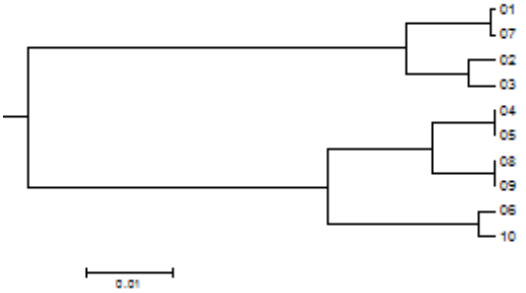

Fig. 2. General similarity of communities. 01- 06 –78 months of seeds storage; 07-10 – 42 months of seeds storage; 01, 07 – Control; 02, 09 – *P. polymyxa* P; 03, 10 – *E. nimipressuralis* 32-3; 04 – mixture of strains; 05 – *R. radiobacter* 10; 06, 08 – *A. vinelandii* 10702

and 0.7829 in control, respectively. We also observed an increase in vigour of tomato seeds obtained from inoculated plants confirming the beneficial effect of the strains.

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