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THE FORMATION OF THE POPULATION OF BACTERIA AND FUNGI IN THE RHIZOSPHERE OF SPRING WHEAT AND WINTER WHEAT

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Abstract. The purpose of the studies carried out in the years 1996-1998 was to establish the composition of bacteria and fungi communities in the soil of spring wheat and winter wheat. Besides, the studies provided the information on quantitative and qualitative composition of amino acids as well as the amount of water soluble sugars in roots exudates of these cereals. The microbiological analysis of 1 g of dry weight of soil coming from the rhizosphere of spring wheat revealed the mean number of 4.94×10^6 bacteria colonies and 45.08×10^3 fungi colonies. In the case of winter wheat, in 1 g of dry weight of the rhizosphere soil 5.07×10^6 bacteria colonies and 28.59×10^3 fungi colonies (mean of three year studies) were found. Besides, winter wheat, through the proper composition of root exudates, created positive nutritional conditions for the growth of microorganisms showing antagonistic effect towards pathogenic fungi. The rhizosphere soil of winter wheat contained 1.5 times more antagonistic bacteria and more than twice antagonistic fungi as compared to spring wheat.

Key words: spring wheat, winter wheat, root exudates, antagonistic microorganisms

I. INTRODUCTION

The quantitative and qualitative composition of microorganism communities in the soil undergoes changes under the effect of various biotic and abiotic factors (Parke 1990; Schroth and Weinhold 1986). Those changes are mainly caused by plants, which through their root exudates and aftercrop residue create the composition of microorganism populations (Funck-Jensen and Hockenhull 1984; Schoruvitz and Zeigler 1989; Sytnik et al. 1977). A particularly important role in the supply of the organic substance is played by plant roots, since their weight constitutes from 50% to 70% of all the after-crop residue. According to the data included in the literature the root mass of spring wheat constituted about 1 dt · ha^{-1} (Pałys 1980/81). In the case of winter wheat, the root mass ranged from 1.5 to 2 dt · ha^{-1}, depending on the rainfall and the soil type (Grzebisz 1991; Pawłowski and Wesołowski 1980/1981).

In the period of vegetation, a great influence on microorganism communities is exerted by root exudates, which are a rich source of amino acids, sugars, organic acids, vitamins, metal ions, phenolic acids and their derivatives (Funck-Jensen and Hockenhull 1984; Pięta 1981; Sytnik et al. 1977). Out of the enumerated compounds, monosaccharides and acid amino acids stimulate the development of phytopathogens, while phenolic compounds, aromatic amino acids and alkaline amino acids have an inhibiting effect (Pięta 1988; Pięta 1994). Root exudates are especially important in increasing the number of propagules of bacteria and antagonistic fungi.

The purpose of the studies was to determine the composition of bacteria and fungi communities in the soil of spring wheat and winter wheat. Besides the qualitative and quantitative composition of amino acids and the amount of water soluble sugars were determined in the root exudates of these cereals.

II. MATERIAL AND METHODS

The studies were carried out in the years 1996-1998 on an experimental field belonging to the Department of Plant and Soil Cultivation of the University of Agriculture in Lublin. The object of the studies was the rhizosphere soil of spring wheat of cv. Sigma and winter wheat cv. Kobra. These varieties were cultivated after soybean as the previous crop.

The experiment was set in a scheme of random blocks in three repetitions, on grey brown podzolic soil made from loess formations, of a second complex of agricultural value (good wheat complex). In each year, the rhizosphere soil was sampled at anthesis. The manner of sampling the rhizosphere soil, and microbiological analysis were carried out according to the method described by Martyniuk et al. (1991). The results were estimated statistically and significant differences were found according to confidence intervals of Tukey (Oktaba 1987).

In each year, the isolates of bacteria (200 isolates of *Bacillus* spp. and 200 isolates of *Pseudomonas* spp.) and all the isolates of saprophytic fungi (from the genera of *Gliocladium* and *Trichoderma*) were used in order to establish their antagonistic effect towards such pathogenic fungi as *Fusarium avenaceum, F. culmorum, Rhizoctonia solani.* In order to determine the antagonistic effect of the examined bacteria on pathogenic fungi a five-degree scale suggested by Martyniuk et al. (1991) and the degree of growth inhibition for phytopathogens provided by Pięta (1999) were used. To estimate the effect of saprophtic fungi on the studied pathogenic fungi a method of biotic series (Mańka 1974; Mańka and Mańka 1992) was used, while the individual effect of antagonistic influence was determined on the basis of the scale given by Mańka and Kowalski (1968).

In order to explain the effect of spring and winter wheat on the formation of fungi and bacteria communities, the studies were performed to establish the quantity of water soluble sugars and the composition of amino acids in the root exudates of those plants. Water solution of root exudates of spring wheat and winter wheat was obtained and chemical analyses were carried out according to the methods described by Pięta (1988).

III. RESULTS

1. Microbiological analysis of the rhizosphere soil of spring wheat and winter wheat

The microbiological analysis of the rhizosphere soil of spring wheat and winter wheat showed differences in numbers of particular microorganism populations (Tab. 1). The total number of bacteria in 1 g of dry weight of soil the rhizosphere of spring wheat was 4.94×10^6 bactaria colonies (mean of three year studies), while the rhizosphere of winter wheat con-

Table 1

The number of bacteria and fungi in the plant rhizosphere

Type of soil	Total number of bacteria (mln/1g d. m. of soil)				Number of bacteria of *Bacillus* genus (mln/1g d. m. of soil)				Number of bacteria of *Pseudomonas* genus (mln/1g d. m. of soil)				Total number of fungi (thous./1g d. m. of soil)			
	1996	1997	1998	mean	1996	1997	1998	mean	1996	1997	1998	mean	1996	1997	1998	mean
Rhizosphere of spring wheat	4.30[a]	6.57[b]	3.96[a]	4.94[a]	1.73[a]	2.40[b]	2.19[b]	2.11[b]	2.18[a]	1.67[a]	1.25[a]	1.70[a]	22.30[a]	47.10[b]	65.85[b]	45.08[b]
Rhizosphere of winter wheat	5.40[b]	4.87[a]	4.96[b]	5.08[a]	1.26[a]	1.78[a]	1.25[a]	1.43[a]	2.75[b]	1.45[a]	2.74[b]	2.31[b]	33.90[b]	17.42[a]	34.47[a]	28.60[a]

Means in columns differ significantly ($P \leq 0.05$), if they are not marked with the same letter.

tained 5.07×10^6 bacteria colonies. The number of bacteria of *Bacillus* spp. in 1 g of dry weight of the soil ranged from 1.25×10^6 to 2.40×10^6 colonies (Tab. 1). Those bacteria were more numerous in 1 g of dry weight of the rhizosphere soil of spring wheat (mean 2.10×10^6), while being less numerous in winter wheat rhizosphere (mean 1.43×10^6). A reverse relationship was observed in numbers of *Pseudomonas* spp. A lower number of those bacteria was found in spring wheat rhizosphere (mean 1.70×10^6), while a higher number was observed in the rhizosphere of winter wheat (mean 2.31×10^6 in 1 g of dry weight of soil). The total number of fungi in 1 g of dry weight of soil in particular years of studies ranged from 17.42×10^3 to 65.85×10^3 colonies (Tab. 1). The rhizosphere of spring wheat contained nearly twice as many fungi colonies than the rhizosphere of winter wheat (Tab. 1).

The mycological analysis of the rhizosphere soil of examined plant species revealed totally 1218 fungi colonies belonging to 44 species (Tab. 2). Among the pathogenic fungi, species of the genus *Fusarium* were the most often isolated. The most representative proved to be *F. culmorum*, whose isolates in the rhizosphere of spring wheat and winter wheat constituted 7% and 3.4% of all the isolates respectively. Also, *F. oxysporum* was frequently isolated and its isolates in the rhizosphere of spring wheat constituted 4.7%, while in winter wheat 3.3% of all the isolates. Besides, other pathogenic fungi such as *F. solani* and *Rhizoctonia solani* were isolated from the rhizosphere of the discussed plant species. The former species was isolated both from the rhizosphere of spring wheat and winter wheat, while *R. solani* only in the case of spring wheat (Tab. 2).

The mycological analysis of the rhizosphere soil of the examined plant species gave numerous fungi isolates from the genera of *Gliocladium, Trichoderma* and *Penicillium.* The genus of *Penicillium* was represented by 17 species, *Gliocladium* spp. by 2 species (*G. catenulatum* and *G. roseum*), while *Trichoderma* spp. by 5 species (*T. hamatum, T. harzianum, T. koningii, T. pseudokoningii, T. viride*) (Tab. 2). More isolates of those fungi were obtained from winter wheat than from spring wheat.

Within *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp. and *Trichoderma* spp., the isolates characterized by

Table 2

Fungi isolated from the plant rhizosphere

Fungus species	Number of isolates							
	rhizosphere of spring wheat				rhizosphere of winter wheat			
	1996	1997	1998	total	1996	1997	1998	total
Acremonium kiliense Grutz			5	5		3		3
Acremonium roseum (Oud.) W. Gams			7	7	3		4	7
Acremonium strictum W. Gams	3	16		19				
Alternaria alternata (Fr.) Keissler	2			2				
Chaetomium piluliferum Daniels		2		2	3			3
Chrysosporium pannorum (Link) Hughes	2	1	4	7	10	8	25	43
Cladosporium cladosporioides (Fres.) de Vries	1		1	2		1		1
Fusarium culmorum (W. G. Sm.) Sacc.	14	10	12	36	8	9	7	24
Fusarium equiseti (Corda) Sacc.	6	3	4	13	7	8	5	20
Fusarium oxysporum Schl.	7	5	12	24	7	7	9	23
Fusarium solani (Mart.) Sacc.	3		4	7	4	1	1	6
Gliocladium catenulatum Gilman et Abbott	4	2		6	5	3	3	11
Gliocladium roseum Bainier	4	3	5	12	6	5	4	15
Mucor mucedo Mich. ex St. Am.	2	4	1	7	21	10	11	36
Penicillium brevi-compactum Dierckx	3	4	6	13	10	18	12	40
Penicillium decumbens Thom	12	3	12	27	15	15	8	38
Penicillium fellutanum Biourge	10		14	24	17	4		21
Penicillium frequentans Westling		12		12		15		15
Penicillium funiculosum Thom	20	10	26	56	10	12	16	38
Penicillium janthinellum Biourge	8	4	6	18	22	11	12	45
Penicillium lividum Westling		1		1			2	2
Penicillium meleagrinum Biourge		1	1	2	2			2
Penicillium nigricans Bainier ex Thom		5		5	3		2	5
Penicillium paxilli Bainier	1	2	2	5	2	1	3	6
Penicillium purpurescens (Sopp) Raper et Thom	3	1	2	6	3	1	1	5
Penicillium purpurogenum Stolk		5		5		3		3
Penicillium roseo-purpureum Dierckx		2		2	2			2
Penicillium velutinum Ramirez et Martinez	3	8		11	12	4	2	18
Penicillium verrucosum Dierckx *var. cyclopium* (Westling)	4		5	9	2	3	1	6
Penicillium verrucosum Dierckx *var. verrucosum* Samson, Stolk et Hadlok	10	1	9	20	11	10	11	32
Penicillium verruculosum Peyronel	4			4		2		2
Periconia macrospinosa Lef. et Johnson	1			1	2			2
Phoma eupyrena Sacc.	26	6		32	3	4		7
Rhizoctonia solani Kuhn		6		6				
Rhizopus nigricans Ehrenberg		3		3	4	3	5	12
Spicaria violacea Abbott		3		3				
Stemphylium botryosum Wallroth	2			2	1		2	3
Torula herbarum (Pers.) Link ex Fr.	7	8	14	29	12	6	17	35
Trichoderma hamatum (Bon.) Bain		1		1	17	8	9	34
Trichoderma harzianum Rifai	5	4	2	11	6	13	9	28
Trichoderma koningii Oud.	14	10	4	28	21	19	11	51
Trichoderma pseudokoningii Rifai	1	5		6	6	7	8	21
Trichoderma viride Pers. ex S. F.Gray	11	8	6	25	14	10	15	39
Verticillium tenerum Ness		3		3			1	1
Total	193	156	164	513	265	224	216	705

Table 3

Antagonistic microorganisms isolated from the plant rhizosphere

Bacteria and fungi	Number of isolates	
	rhizosphere of spring wheat	rhizosphere of winter wheat
Bacillus spp.	78	100
Pseudomonas spp.	116	183
Total bacteria	194	283
Gliocladium catenulatum Gilman et Abbott	6	11
Gliocladium roseum Bainier	12	15
Trichoderma hamatum (Bon.) Bain	1	34
Trichoderma harzianum Rifai	11	28
Trichoderma koningii Oud.	28	51
Trichoderma pseudokoningii Rifai	6	21
Trichoderma viride Pers. ex S. F. Gray	25	39
Total fungi	89	199

antagonistic effect towards *F. avenaceum, F. culmorum* and *R. solani* were separated. Frequency of the occurrence of antagonistic microorganisms in the rhizosphere of spring wheat and winter wheat was different (Tab. 3). In the case of *Gliocladium* spp. and *Trichoderma* spp. all the isolates from the studied rhizosphere soils turned out to be antagonists. On the other hand, the antagonistic bacteria of *Bacillus* spp. constituted 14% on the average, while *Pseudomonas* spp. 25% of all the isolates. In the rhizosphere of winter wheat much more antagonistic microorganisms were found than in spring wheat. The rhizosphere soil of winter wheat contained 1.5 times more antagonistic bacteria and more than twice as many antagonistic fungi than in spring wheat (Tab. 3).

2. Chemical analysis of root exudates of spring wheat and winter wheat

Chemical analysis of root exudates of spring wheat and winter wheat revealed differences in the quantitative and qualitative composition of free amino acids occurring in 1 ml of water solution (Tab. 4). In the case of winter wheat, the amount of free amino acids exuded by the roots was 0.484 mg/ml of the solution. Spring wheat roots exuded only 0.148 mg/ml (Tab. 4). Out of 15 free amino acids present in root exudates of analyzed plant species, the dominant ones were aspartic acids and glutanic acid. Their proportion in the root exudates of spring wheat and winter wheat was 39.8% and 39% of the total quantity of free amino acids, respectively.

The roots of examined plants also exuded alkaline amino acids such as lysine, hystidine and arginine. A greater proportion of those amino acids (22.5%) was found in the exudates of winter wheat roots than in spring wheat (9.4% of total amount of the discussed compounds).

Besides, the root exudates contained free aromatic amino acids, including tyrosine and phenylalanine. More aromatic amino acids were exuded by winter wheat roots (21%), while less by the roots of spring wheat (12% of total amount of free amino acids).

Table 4

The contents of free amino acids and sugars (in mg/ml) in the exudates of plant roots

Plants	Sour amino acids		Aromatic amino acids		Alkaline amino acids			treonine	serine	glycine	alanine	valine	metio-nine	isoleu-cine	leucine	Total amino acids	Water souble sugars
	aspartic acid	glutam. acid	tyrosine	phenyl-alannine	lysine	histidine	arginine										
Spring wheat	0.029	0.030	0.009	0.010	0.004	0.006	0.004	0.011	0.001	0.011	0.006	0.010	0.007	0.003	0.007	0.148	trace
Winter wheat	0.091	0.099	0.086	0.017	0.037	0.046	0.026	0.017	0.014	0.009	0.010	0.013	0.003	0.012	0.004	0.484	0.117

The other amino acids (treonine, serine, glycine, alanine, valine, methionine, isoleucine, leucine) which were present in root exudates of winter wheat constituted 0.082 mg/ml of the solution, while those in spring wheat made up 0.056 mg/ml of the solution.

The chemical analysis showed that apart from amino acids the roots of spring wheat exuded trace quantities of water soluble sugars. The total content of those compounds in the root exudates of winter wheat was 0.117 mg/ml of the water solution (Tab. 4).

IV. DISCUSSION

Results of the studies pointed at different composition of bacteria and fungi in the rhizosphere soil of the examined plants. From the point of view of a phytopathologist, the rhizosphere soil of winter wheat had a better composition of microorganisms, since it contained a lower total number of fungi, and a higher total number of bacteria than the rhizosphere soil of spring wheat. Besides, winter wheat created favourable conditions for the growth of antagonistic bacteria. This is proved by particularly numerous occurrence of antagonistic bacteria of *Bacillus* spp. and *Pseudomonas* spp. in the rhizosphere of that plant species. According to Keel (1992) and Weller (1988), bacteria *Pseudomonas* spp. are capable of active settlement of plant roots, and because of this they effectively compete with pathogens for nutritional elements available in root exudates, in this way becoming a factor of biological control of pathogens.

The antagonistic fungi *Gliocladium* spp. and *Trichoderma* spp. were found in the rhizosphere of the discussed plant species. Winter wheat had a stimulating effect on the growth of *Gliocladium* spp. and *Trichoderma* spp., since in the rhizosphere of this plant species twice as many isolates of those fungi were found than in the case of spring wheat. Those antagonistic fungi significantly limit the population of phytopathogens living in the soil (Łacicowa and Pięta 1985; Łacicowa and Pięta 1989; Papavizas 1985).

According to Funck-Jensen and Hockenhull (1984) free amino acids in root exudates constitute the basic source of nourishment for the microorganisms in rhizosphere. The results of the chemical analysis made it possible to conclude that

three times more free amino acids were found in the root exudates of winter wheat than in spring wheat. It should be supposed that a greater quantity and the proper composition of root exudates of winter wheat create favourable nutritional conditions for the development of saprophytic microorganisms showing the antagonistic effect. Both antagonistic bacteria (*Bacillus* spp., *Pseudomonas* spp.) and antagonistic fungi (*Gliocladium* spp., *Trichoderma* spp.) limit the growth of pathogenic fungi living in the soil environment, which in turn determines the phytosanitary state of the soil (Hadar et al. 1984; Keel 1992; Papavizas 1985).

V. CONCLUSIONS

1. The composition of microorganism population in the rhizosphere soil of winter wheat suggests a more favourable effect of root exudates of this plant on the growth of antagonists than that of spring wheat root exudates.
2. The rhizosphere of winter wheat contained more antagonistic bacteria (*Bacillus* spp., *Pseudomonas* spp.) and antagonistic fungi (*Gliocladium* spp., *Trichoderma* spp.) than the rhizosphere of spring wheat.
3. Winter wheat plants contribute to a greater biological activity of the soil and improve the phytosanitary condition of the soil.

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KSZTAŁTOWANIE SIĘ POPULACJI BAKTERII I GRZYBÓW W RYZOSFERZE PSZENICY JAREJ I PSZENICY OZIMEJ

STRESZCZENIE

Celem badań było określenie kształtowania się populacji bakterii oraz grzybów w ryzosferze pszenicy jarej i pszenicy ozimej. W wyniku prezentowanych badań stwierdzono, że pszenica ozima miała korzystny wpływ na ogólną liczbę bakterii, a w tym szczególnie na bakterie z rodzaju *Pseudomonas*. Ponadto w glebie ryzosferowej pszenicy ozimej było mniej o 37% kolonii grzybów, aniżeli u pszenicy jarej. Pszenica jara miała mniejszy wpływ na rozwój bakterii, natomiast stymulowała wzrost liczebności grzybów. Poprzez odpowiedni skład wydzielin korzeniowych pszenica ozima stwarzała dogodne warunki pokarmowe dla rozwoju mikroorganizmów o antagonistycznym oddziaływaniu względem fitopatogenów.

W glebie ryzosferowej pszenicy ozimej było 1,5 raza więcej bakterii antagonistycznych i ponad dwukrotnie więcej grzybów antagonistycznych, aniżeli u pszenicy jarej.