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Effect of biofertilizers on yield and quality of long-fibred flax and cereal grains

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Abstract. Application of biofertilizers provides the implementation of biological mechanisms of plant nutrition, growth promotion and protection. These are arguments for the use of biofertilizers as elements for nutrient management in organic agriculture, along with low cost and environmental safety. Azobacterin and Kaliplant were developed in Belarus. Natural N_2-fixing bacteria are acting agents of Azobacterin. Kaliplant contains a natural strain of K-mobilizing bacteria. Both strains possess P-solubilization activity. The effects of biofertilizers on crop yield and quality were studied in field experiments on Luvisol soils. The contribution of biofertilizers for the crop yield increment varied in range from 8–30%. Azobacterin applications were most effective for barley and long-fibred flax. Kaliplant inoculations were mostly profitable for winter rye and winter triticale. Biofertilizers positively influenced crop production quality. Reliable increase of protein content and the improvement of amino acid composition in cereal grains were observed.

Key words: biofertilizers, Azobacterin and Kaliplant, *Azospirillum brasilense, Bacillus circulans,* crop yield, production quality

INTRODUCTION

Application of biofertilizers provides effective implementation of biological mechanisms of plant nutrition, growth promotion and protection (Okon, 1982; Bashan & Levanony, 1990; Bashan et al., 1995; Boddey & Dobereiner, 1995). These are important arguments for the use of biofertilizers as prospective elements for nutrient management in organic agriculture. Low cost and safety for the environment make fertilizers advantageous as an alternative to mineral fertilizers.

Biofertilizers Azobacterin and Kaliplant were developed in the Belarusian Research Institute for Soil Science and Agrochemistry. Natural diazotrophic bacteria strain *Azospirillum brasilense* B-4485 is the acting agent of Azobacterin (Nesterenko et al., 1997). Kaliplant contains the natural K-mobilizing strain *Bacillus circulans* BIM B-376D, which is capable of releasing part of K-cations from hardly available potassium forms in soil (Mikhailouskaya et al., 2007).

Biological N_2-fixation by diazotrophic bacteria is a spontaneous process if soil N is limited and adequate C sources are available. It is well documented that diazotrophic bacteria significantly increase plant vegetative growth and grain yield (Tien et al., 1979; Okon & Kapulnik, 1986; Okon & Itzigsohn, 1995). The ability of these bacteria to contribute to yields in crops is only partly a result of biological N_2-fixation. The mechanisms involved have a significant plant-growth promotion potential. Economic and environmental benefits can include increased income from reduced fertilizer cost, reduced leaching of NO_3^-- N to ground water as well as reduced emission of N_2O greenhouse gas, the global warming effect of which is 300 times more than CO_2 (Kennedy et al., 2004).

Improving K-nutrition in agricultural plants is also an important task. Taking into account significant total potassium reserve in Luvisol soils, widespread in Belarus, the use of K-mobilizing bacteria may be considered as an alternative pathway of K-nutrition. Soil microorganisms and their metabolites play key roles in the processes of transformation of soil minerals providing the release of potassium and other elements from aluminosilicates (Berthelin, 1983; Glazovskaya, 1984; Ferris et al., 1989; Bennett, 1991; Ullman et al., 1996). Microorganisms are capable of acceleration or inhibition of aluminosilicates' dissolution (Ullman et al., 1996; Barker, 1998) as well as biogenic mineral formation (Ferris et al., 1989; Huang, 1991).

The aim of this study was to quantify the responses of the yield and quality of cereal grains and straw of long-fibered flax to biofertilizers Azobacterin and Kaliplant. The experimental data may be helpful in considering the possible use of biofertilizers as an element of nutrient management in organic agriculture.

MATERIALS AND METHODS

Effects of biofertilizers Azobacterin and Kaliplant on crop yields and its quality were studied in field experiments on Luvisol loamy sand and sandy loam soils characterized by typical fertility status for the main regions of Belarus.

Azobacterin was applied by seed treatment directly before sowing. *Azospirillum brasilense* B-4485 titres in peat preparation were as follows: for barley inoculation 3–4×10^9 CFU g^{-1}, long-fiber flax 10^8–10^9 CFU g^{-1}. Agrochemical properties of tested soils (humus, pH_{KCl}, mobile K_2O and P_2O_5) are presented in Tables 1–2.

Kaliplant in liquid preparation form (titre 4–6 10^9 CFU ml^{-1}) was used as the spray treatment at stem elongation growing stages (GS, by Zadoks 31-33). Kaliplant was tested in the long-term field experiment on Luvisol loamy sand soil (Minsk region). Four blocs with different content of exchangeable potassium in soil were prepared: 1st level 95–105, 2nd level 165–186, 3rd level 220–222 and 4th level 270–280 mg K_2O kg^{-1}. Fertilizers (ammonium nitrate, triple superphosphate, potassium chloride) were introduced annually under presowing cultivation of soil.

A conventional one- or two-factorial dispersive statistical analysis for the experimental data was carried out using the MS Excel program (Clever, 2001). The test of statistically significant differences at the Fisher criterion (with the probability < 0.05) was used to analyse the mean differences. Average straw number was estimated as the integral value index of the following parameters: length, base content, strength, appropriateness, color and stem diameter. Chemical, physical and organoleptic methods were used for the quantitative determination of indicated parameters. In accordance with State Standard, every parameter number was found with the help of special tables. Average straw number represents the sum of all parameter numbers (Trush, 1986).

RESULTS AND DISCUSSION

The field experiments revealed a significant contribution of Azobacterin and Kaliplant to the yield increase of the field crops, which vary in range from 8–30% of control value depending on crop, soil properties and weather conditions.

Soil treatments included PK and $N_{30}PK$ fertilization in both experiments. Azobacterin application on Luvisol loamy sand soil during 1996–2000 resulted in statistically sound grain responses 0.27 and 0.48 t ha^{-1} (Table 1). Comparison of biofertilizer effectiveness for barley inoculation on two Luvisol varieties revealed the role of soil fertility status. Application of Azobacterin was more effective on Luvisol sandy loam soil with higher fertility status, with grain yield responses 0.54 and 0.65 t ha^{-1} (Table 1).

Table 1. The effect of Azobacterin on barley grain yields and quality on Luvisol soils.

Crop	Luvisol texture	Treatment	Grain yield, t ha^{-1}			Protein content in grain, %	
			Control	Azo-bacterin	Response	Control	Azo-bacterin
Barley 1996–2000	loamy sand	PK	3.11	3.38	0.27*	8.5	9.3*
		$N_{30}PK$	3.68	4.16	0.48 *	9.8	11.5*
			LSD_{05} 0.21			LSD_{05} 0.56	
Soil properties: pH 6.0–6.2; humus 1.8–1.96%; P_2O_5 158–176, K_2O 143–156 mg kg^{-1}							
Barley 1996-1998	sandy loam	PK	4.46	5.00	0.54 *	11.1	11.9*
		$N_{30}PK$	4.64	5.29	0.65 *	11.6	12.1
Soil properties: pH 6.1–6.3; humus 2.1–2.3%; P_2O_5 360–380, K_2O 230–250 mg kg^{-1}							
				LSD_{05} 0.53			LSD_{05} 0.7

* Responses are significant at $P = < 0.05$

Table 2. The mean effects of Azobacterin on straw yield and quality of long-fibred flax on Luvisol sandy loam soil (1998–2000).

Treatment	Flax retted straw, t ha^{-1}			Long fiber, t ha^{-1}			Straw number (quality index)	
	Control	Azo-bacterin	Response	Control	Azo-bacterin	Res-ponse	Cont-rol	Azo-bacterin
PK	4.46	5.42	0.96*	0.72	1.08	0.36*	1.58	2.17
$N_{15}PK$	5.19	6.48	1.29*	1.01	1.34	0.33*	1.92	2.17
$N_{30}PK$	6.13	-	-	1.17	-	-	2.00	-
		LSD_{05} 0.41			LSD_{05} 0.16			
Soil properties: pH 5.4–5.7; humus 1.6–1.7%; P_2O_5 150–180, K_2O 160–180 mg kg^{-1}								

* Responses are significant at $P = < 0.05$

It can be considered that more effective realization of plant genetic and diazotrophs could be achieved on fertile soil. Possible reducing of N-rates for barley growing may account for approximately 30 kg ha^{-1} because on both soils comparable yields were obtained at treatments $N_{30}PK$ and PK+Azobacterin (Table 1).

Field experiment data showed also the possibility of partial replacement of mineral N fertilization for long-fibred flax by application of Azobacterin (Table 2).

Flax needs a relatively low level of nitrogen nutrition. In fact, it is known that excess nitrogen affects flax yield and fiber quality negatively (Trush, 1986). In our experiment the N requirement was partially supplied due to diazotrophs application. No nitrogen deficit was observed for flax plants due to seed inoculation in combination with PK-fertilizing compared with $N_{15}PK$ treatments. The effects of inoculation on the yield of flax straw and long fiber were slightly higher than the effect of N 15 kg ha^{-1} (Table 2). The use of biofertilizer resulted in significant improvement of flax fiber and straw quality. The best quality index of straw (2.17) was obtained due to the application of Azobacterin on backgrounds of $P_{60}K_{90}$ and $N_{15}P_{60}K_{90}$ fertilization (Table 2). Implementation of biological management of flax nutrition may be profitable for farmers because the cost of biofertilizer inoculation is lower than comparable N fertilizer rate treatment.

The grain yield responses of winter rye and winter triticale to top dressing with Kaliplant (200–2007) are shown in Tables 3–4.

Table 3. Effect of Kaliplant on the yield and quality of winter rye grain on Luvisol loamy sand soil.

	Without fertilization		$N_{90}P_{30}$			$\Sigma AA_{critical}$
	Yield, t ha^{-1}	Response, t ha^{-1}	Yield, t ha^{-1}	Response, t ha^{-1}	Protein in grain, %	mg g^{-1} of protein
			1st level, K_2O 105 mg kg^{-1}			
Control	1.25	-	2.35	-	7.7	72.5
Kaliplant	2.24	0.99*	2.89	0.54*	8.2*	83.2*
			2nd level, K_2O 186 mg kg^{-1}			
Control	1.69	-	2.90	-	8.3	74,1
Kaliplant	2.43	0.74*	3.56	0.66*	8.7*	82.8*
			3rd level, K_2O 222 mg kg^{-1}			
Control	1.76	-	3.17	-	8.5	75.5
Kaliplant	2.13	0.37*	3.58	0.41*	9.0*	82.2*
			4th level, K_2O 280 mg kg^{-1}			
Control	1.98	-	2.88	-	8.5	86.0
Kaliplant	2.20	0.22*	3.32	0.44*	8.5	85.1
LSD_{05} A (K_2O)	0.24		0.28		0.35	0.94
B (Kaliplant)	0.17		0.20		0.27	1,2

* Responses are significant at *P*= <0.05

$\Sigma AA_{critical}$ – critical amino acids content, mg g^{-1} of protein

It was found that the introduction of Kaliplant provides statistically sound responses of cereal grain yields in a wide range of K_2O content in soil 95–280 mg kg^{-1}. However much higher grain yield responses of winter rye (0.99–0.74 t ha^{-1}) to Kaliplant dressing were observed under a relative deficit of mobile potassium in soil, at 1st and 2nd levels of soil K-supply (Table 3). Reliable, but significantly lower winter rye grain yield responses (0.37–0.22 t ha^{-1}) were obtained under sufficient soil K supply K_2O 222–280 mg kg^{-1}.

Table 4. Effect of Kaliplant on the yield and quality of winter triticale grain on Luvisol loamy sand soil.

	Without fertilization		$N_{90}P_{30}$			Lysine,
	Yield, t ha^{-1}	Response, t ha^{-1}	Yield, t ha^{-1}	Response, t ha^{-1}	Protein in grain, %	g kg^{-1} of grain
			1st level, K_2O 95 mg kg^{-1}			
Control	1.75	-	2.69	-	13.4	3.2
Kaliplant	2.35	0.60*	3.46	0.77*	14.2*	4.3*
			2nd level, K_2O 165 mg kg^{-1}			
Control	2.59	-	3.80	-	13.4	4.2
Kaliplant	3.07	0.48*	4.29	0.49*	14.7*	4.5*
			3rd level, K_2O 220 mg kg^{-1}			
Control	2.50	-	4.25	-	13.8	4.3
Kaliplant	2.84	0.34*	4.59	0.34*	14.6*	4.7*
			4th level, K_2O 270 mg kg^{-1}			
Control	2.55	-	4.26	-	14.2	4.7
Kaliplant	2.92	0.37*	4.53	0.27*	14.9*	4.7
LSD_{05} A (K_2O)	0.27		0.35		0.49	0.11
B (Kaliplant)	0.19		0.25		0.33	0.08

* Responses are significant at *P*= <0.05

The grain yield increments of winter triticale due to Kaliplant application has also been higher at the first two levels of soil K supply (0.60–0.48 t ha^{-1}) compared to grain yield responses (0.34–0.37 t ha^{-1}) at the 3rd and 4th levels of K supply (Table 4).

The current growing interest of scientists and farmers in biofertilizers is the result of their positive influence on quality of crop production. Our investigations have shown the reliable increase of protein content in rye grain (by 0.4–0.5%) at 1st–3rd levels of soil K supply and in triticale (on 0.7–1.3%) at all tested levels of mobile K content in soil (Tables 3–4). Application of Kaliplant has been followed with sufficient increase in content of the critical amino acids in protein of winter rye grain (Table 3) as well as lysine content in grain of winter triticale (Table 4) thereby improving protein quality at 1st–3rd levels of soil K supply. Application of Kaliplant did not increase the concentration of lysine in triticale grain only in soil with a high level of K_2O 270 mg kg^{-1}.

The ability of Azobacterin and Kaliplant to contribute to crop yield increase is only partly a result of biological N_2-fixation or K-mobilization. The mechanisms involved have a significant plant growth promotion potential, which was quantitatively evaluated in model laboratory experiments. *A. brasilense* was found to increase the root volume by 28%, wet mass – by 45%; *B. circulans* caused the increase of the root volume by 18%, wet mass – by 24% (Mikhailouskaya et al., 2007). Stimulation of root development and root surface area improves the adaptive potential of inoculated plants. Both strains are characterized by P-solubilizing capability that provides them with additional competitive advantages in the rhizosphere. Resistance to pathogens, siderophore production, P-mobilization, enhanced stress resistance, and vitamin production are widely discussed as possible effects of biofertilizers on plants (Tien et al., 1979; Okon & Kapulnik, 1986; Boddey & Dobereiner, 1995; Kennedy et al., 2004). Presumably inoculation may promote crop yields by modifying soil–plant processes so that N, K, P and other nutrients are more completely retained in the plant–soil system, thus reducing the need for fertilizer (Kennedy et al., 2004).

CONCLUSIONS

Long term field studies showed a significant contribution of Azobacterin and Kaliplant for the yield increase of the field crops on Luvisol soils, which vary in range from 8–30% of control value depending on crop and soil fertility. Application of Azobacterin showed also the possibility of mineral fertilization reduction of N 15 kg ha^{-1} for long-fibered flax and N 30 kg ha^{-1} for cereal grains with simultaneous improvement of production quality.

Application of Kaliplant has also been followed by a sufficient increase of protein content in grain of winter rye (on 0.4–0.5%) and in winter triticale (on 0.7–1.3%) as well as with improved protein quality at low and medium levels of soil K supply. The experimental data may be helpful in considering the possible use of biofertilizers as an element of nutrient management in organic agriculture.

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