The effect of flax seed inoculation by *Azospirillum brasilense* on flax yield and its quality

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ABSTRACT

Field experiment demonstrated the benefit resulting from biological soil management including the use of N_2-fixing and growth promoting bacteria *A. brasilense* B-4485 for long-fibred flax. Seed inoculation by *A. brasilense* B-4485 was equivalent to the introduction of 15 kg/ha of N that provided the possibility of partial flax N requirement supply. Treatment *A. brasilense* + $N_{15}P_{60}K_{90}$ is considered to be the most profitable management in regard to flax yield and its quality, and is comparable to $N_{30}P_{60}K_{90}$ treatment. Biological management allows preventing high concentrations of N in soil, excludes lodging and negative effects on flax yield and its quality. Implementation of biological management for flax nutrition may be profitable for both ecology and economy of long-fibered flax growing.

Keywords: flax; *Azospirillum brasilense*; seed inoculation; flax quality

The development of modern ecologically friendly and profitable management for long-fibred flax growing is of a significant importance. Flax needs a relatively low level of nitrogen nutrition. An excess of nitrogen fertilizer was found to negatively affect flax yield and its quality because of lodging and development of diseases (Trush 1986). Relatively low flax requirement in nitrogen was the prerequisite for the experiments with associative N_2-fixing bacteria *Azospirillum brasilense*, which also possess a hormonal effect. Flax nitrogen requirement was supposed to be partially supplied due to *A. brasilense* application.

Diazotrophic bacteria, belonging to *Azospirillum* genera, attract a lot of interest because of their capability of plant growth promotion and mineral and water nutrition improvement (Bashan and Levanony 1990, Bashan et al. 1995, Boddey and Dobereiner 1995). Experiments with cereal crops have demonstrated an evident benefit from *Azospirillum* inoculation (Okon 1982, Reynders and Vlassak 1982, Baldani et al. 1987, Belimov et al. 1995, Boddey and Dobereiner 1995).

Local strain *Azospirillum brasilense* B-4485 possesses a high N_2-fixing activity and a significant hormonal effect. Strain *A. brasilense* B-4485 proved to be an effective inoculant for barley, wheat and perennial grasses (Nesterenko et al. 1995, Mikhailouskaya 1999).

Azospirillum brasilense was found to possess no obligate specificity in respect to the host plant (Bashan and Levanony 1990, Bashan et al. 1995). Therefore testing the effect of *A. brasilense* B-4485 on field grown flax is of a significant interest. The relatively low flax need for nitrogen suggests the possibility to stimulate associative N_2-fixation and growth promotion by means of associative diazotrophic bacteria introduction. The purpose of the experiment was to study the effect of flax seed inoculation by *Azospirillum brasilense* B-4485 on yield and production quality and to evaluate the possibility to reduce the rates of N-fertilizing for flax.

MATERIAL AND METHODS

Field experiments with *Linum usitatissimum* L., cultivar Niva were carried out on Luvisol sandy loam soil in 1998–2000. Arable layer of soil was characterized by the following agrochemical parameters: humus content 1.55–1.76% (Tjurin method), pH(KCl) 5.4–5.7, P_2O_5 150–180 mg/kg, K_2O 160–180 mg/kg.

Fertilizer treatments in the field experiment (NPK, kg/ha) were as follows: $N_0P_{60}K_{90}$, $N_{15}P_{60}K_{90}$, $N_{30}P_{60}K_{90}$, $N_{45}P_{60}K_{90}$. Plot square was equal to 24 m^2, 4 replication. Before sowing flax seeds were inoculated by peat preparation of *Azospirillum brasilense* B-4485 (10^8–10^9 viable cells/g).

The evaluation of population density of *A. brasilense* on the roots was performed with the use of Pen 2600 spontaneous mutant (Zvyagintsev 1991). Modifying Nfb medium was used (Dobereiner et al. 1976).

Dynamics of root biomass accumulation was estimated (dry matter per 20 plants, g) at the following development phases: stem extension, flowering and yellow ripeness.

Straw quality was estimated with the use of quality index, which is an integral quantitative evaluation of the following parameters: length, bast content, strength, appropriateness, color and stem diameter (Trush 1986).

The weather conditions in 1999 and 2000 were characterized by the lack of precipitation. In 1998 excess rainfall was observed.

RESULTS AND DISCUSSION

Introduced bacteria *Azospirillum brasilense* B-4485 was found to colonize well the flax roots.

At the background of $P_{60}K_{90}$-fertilization bacteria abundance achieved 5–6 × 10^4 colony forming units (CFU) at flowering phase and 1–3 × 10^4 CFU (per g of fresh roots) at yellow ripeness phase. At the background of $N_{15}P_{60}K_{90}$-fertilization the population density was approximately 1.5–2.0 times lower.

Flax seed inoculation by *A. brasilense* caused the stimulation of plant roots development. The root mass of inoculated plants was 1.3–1.6 times higher than that for not inoculated treatments (Table 1). A more intensive root development as affected by inoculation was observed at intensive growth and yellow ripeness phases.

Seed inoculation by *A. brasilense* and the application of mineral fertilizers resulted in the increase of the field grown flax yield. High and comparable yields of straw, retted straw, flaxseeds (0.89 and 0.87 t/ha) and long fibre (1.34 and 1.17 t/ha) were obtained as a result of N_{15}PK + *A. brasilense* and N_{30}PK treatments (Tables 2–4). In regard to the yields, N_{15}PK + *A. brasilense* and N_{30}PK treatments were considered as the most profitable for field grown flax.

The treatment PK + *A. brasilense* demonstrated no nitrogen deficit for flax plants compared with N_{15}PK-fertilization in respect of flax yield and stem length (Tables 2–4). There is a possibility to grow flax without N-fertilizer, however top yields of flax were obtained due to N_{15}PK + *A. brasilense* as well as N_{30}PK application. Balanced K and P nutrition is one of the most important factors for the success of seed inoculation because potassium and phosphorus concentrations strongly affect N_2-fixing bacteria survival, their propagation and activity in the root zone. Seed inoculation at the background of PK-fertilization ($P_{60}K_{90}$ + *A. brasilense* treatment) resulted in the increase of long fibre yield by 1.5 times and flaxseeds by 33% compared with $P_{60}K_{90}$ treatments without bacteria introduction (Table 4).

Yields of flax production depended on N-fertilizer rates. On Luvisol sandy loam soil optimum rates of N-fertilizer lie in margins 15–30 kg/ha. The application of 45 kg/ha of N did not provide any increase of straw yield because of lodging. In this case the reduction of retted straw yield as well as flaxseeds and long fibre was observed (Tables 2–4).

Table 1. Effect of *A. brasilense* B-4485 on the development of flax roots (dry matter, g/20 plants) (1998–2000)

Treatment	Development phase					
	stem extension		flowering		yellow ripeness	
	control	*A. brasilense*	control	*A. brasilense*	control	*A. brasilense*
PK	1.71	2.73	3.64	4.87	4.25	6.54
N_{15}PK	1.89	2.83	3.97	5.34	4.60	7.39
N_{30}PK	2.31	–	4.55	–	5.40	–
N_{45}PK	2.09	–	4.22	–	4.95	–
$LSD_{0.05}$	0.09–0.10		0.10–0.11		0.18–0.19	

P – 60 kg/ha, K – 90 kg/ha

Table 2. Effect of *A. brasilense* B-4485 on flax straw and retted straw yields (1998–2000)

Treatment	Flax straw (t/ha)		Flax retted straw (t/ha)	
	yield	response	yield	response
Without inoculation				
PK	5.39	–	4.46	–
$N_{15}PK$	6.35	0.96*	5.19	0.73*
$N_{30}PK$	7.39	2.00*	6.13	1.67*
$N_{45}PK$	6.77	1.38*	5.59	1.13*
Inoculation by *A. brasilense*				
PK	6.56	1.17**	5.42	0.96**
$N_{15}PK$	7.81	1.46**	6.48	1.29**
$LSD_{0.05}$	0.22–0.41			

P – 60 kg/ha, K – 90 kg/ha, *yield response as a result of N-fertilizer application, **yield response as a result of inoculation by *A. brasilense*

High stem length is the main precondition of fibre quality, for long stems are characterized by higher fibre content. The lack of nitrogen resulted in the formation of low stems (Trush 1986). The application of N_2-fixing bacteria at the backgrounds of PK and $N_{15}PK$ fertilization demonstrated the highest stem length, which was comparable with that of $N_{30}PK$ treatment (Table 5). Seed inoculation provided the achievement of high stem length as well as a possibility to reduce N-fertilizer expenses. PK-fertilizing + *A. brasilense* resulted in 7 cm stem length response and contributed to high stem length of flax without N-fertilizer use (Table 5).

Straw quality index was used, representing the integral quantitative evaluation of following parameters: length, bast content, strength, appropriateness, color and stem diameter. The best quality indexes of flax straw (2.17) were obtained due to biological soil management at the backgrounds of PK and $N_{15}PK$-fertilizing (Table 4). Optimal N-rate was found to be 30 kg/ha; quality index however did not exceed 2.00 (Table 5). The increase of N-rate up to 45 kg/ha negatively affected quality index (1.75) as well as the unbalanced PK treatment (1.58) did (Table 5).

Field experiment gave the possibility to compare the effectiveness of different soil managements for flax growth. Data obtained in 1998–2000 demonstrated the evident benefit of *A. brasilense* B-4485 application for flax growing. *A. brasilense* + $N_{15}PK$ treatment is considered as the most profitable management for flax yield, quality improvement and reduction of N-fertilizers consumption (Tables 2–5). High and comparable yields of flax production were obtained as a result of PK + *A. brasilense* and $N_{15}PK$ treatments, as well as $N_{15}PK$ + *A. brasilense* and $N_{30}PK$. Top yields of flax seeds and long fibre were obtained as a result of $N_{15}PK$ + *A. brasilense* application. Seed inoculation was equivalent to the introduction of 15 kg/ha of N at background of optimal rates of phosphorus and potassium. Biological management allows preventing high concentrations of N in soil, which negatively affects

Table 3. Effect of *Azospirillum brasilense* B-4485 on flax seeds yield (t/ha) (1998–2000)

Treatment	Yield	Response
Without inoculation		
PK	0.60	–
$N_{15}PK$	0.76	0.16*
$N_{30}PK$	0.87	0.27*
$N_{45}PK$	0.81	0.21*
A. brasilense		
PK	0.80	0.20**
$N_{15}PK$	0.89	0.13**
$LSD_{0.05}$	0.035–0.050	

P – 60 kg/ha, K – 90 kg/ha, *yield response as a result of N-fertilizer application, **yield response as a result of inoculation by *A. brasilense*

Table 4. Effect of fertilizers and *A. brasilense* B-4485 on fibre yield (t/ha) (1998–2000)

Treatment	Total		Long fibre			
	control	*A. brasilense*	control	*A. brasilense*	response	
					N-rate	inoculation
PK	1.31	1.72	0.72	1.08		0.36
N15PK	1.62	2.07	1.01	1.34	0.29	0.33
N30PK	1.87		1.17		0.16	
N45PK	1.62		0.88		–0.29	
$LSD_{0.05}$	0.21		0.16			

Table 5. Effect of fertilizers and *A. brasilense* B-4485 on quality of flax straw (1998–2000)

Treatment	Technical stem length (cm)	Strength (kg)	Quality index
Without inoculation			
PK	75.0	25.7	1.58
N_{15}PK	81.2	28.0	1.92
N_{30}PK	82.0	28.3	2.00
N_{45}PK	81.8	26.3	1.75
Inoculation by *A. brasilense*			
PK	82.3	29.7	2.17
N_{15}PK	82.4	29.6	2.17
$LSD_{0.05}$	3.8	0.24	

flax yield and its quality (Trush 1986). There was no lodging observed due to biological soil management. Inoculation experiments have shown that flax nitrogen demand may be partially supplied due to the introduction of associative diazotrophic bacteria, which contributes to the improvement of flax nutrition by means of hormonal effect and N_2-fixation associated with flax roots. Biological soil management favors the reduction of N-fertilizer consumption for flax growing by 15 kg/ha. The application of *A. brasilense* B-4485 is an alternative technique to achieve the same results as the introduction of N-fertilizer at the rate of 15 kg/ha.

The expenses for fertilization at $N_{30}P_{60}K_{90}$ and $N_{15}P_{60}K_{90}$ + *A brasilense* treatments were similar. Economic advantages provided a high response to inoculation, as for instance, the cost of retted straw response was equal to 133 USD per hectare, harvesting expenses were 32.5 USD per hectare and the final economical profit due to biological management reached 100 USD per hectare.

REFERENCES

Baldani V.L.D., Baldani J.I., Dobereiner J. (1987): Inoculation of field-grown wheat with *Azospirillum* spp. in Brazil. Biol. Fert. Soils, *4*: 37–40.

Bashan Y., Levanony H. (1990): Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. Can. J. Microbiol., *36*: 591–608.

Bashan Y., Ream Y., Levanony H., Sade A. (1995): Nonspecific responses in plant growth, yield and root colonization of noncereal crop plants to inoculation with *Azospirillum brasilense* Cd. Can. J. Bot., *67*: 1317–1324.

Belimov A.A., Kunakova A.M., Vasilyeva N.D., Gruzdeva E.V. et al. (1995): Relationship between survival rates of associative nitrogen-fixers on roots and yield response of plants to inoculation. FEMS Microbiol. Ecol., *17*: 187–196.

Boddey R.M., Dobereiner J. (1995): Nitrogen fixation associated with grasses and cereals: Recent progress and perspectives for the future. Fert. Res., *42*: 241–250.

Dobereiner J., Marriel J.E., Nery M. (1976): Ecological distribution of *Spirillum lipoferum* Baijerink. Can. J. Microbiol., *22*: 1464.

Methods of chemical analyses of soil (1975): Science, Moscow: 62–63, 206–207. (In Russian)

Mikhailouskaya N. (1999): Associative N_2-fixation and N_2-fixing bacteria application for barley and forage grasses. Int. Agr. J., Minsk, *11*: 20–22. (In Russian)

Nesterenko V., Mikhailouskaya N., Barashenko T. (1995): Influence of associative N_2-fixing bacteria on barley growth. Soil Sci. Fert. Appl., Minsk, *23*: 261–167. (In Russian)

Okon Y. (1982): Azospirillum: Physiological properties, mode of association with roots and its application for the benefit of cereal and forage grass crops. Israel J. Bot., *31*: 214–220.

Reynders L., Vlassak K. (1982): Use of *Azospirillum brasilense* as a biofertilizer in intensive wheat cropping. Plant Soil, *66*: 217–233.

Trush M.M. (1986): Practical Recommendation for Intensive Technology for Long-Fibered Flax Growing. Agropromizdat, Moscow. (In Russian)

Zvyagintsev D.G. (1991): Methods for Soil Microbiology and Biochemistry. Moscow State University, Moscow. (In Russian)

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