Effects of Foliar Application with Effective Microorganisms on Leaf Metabolism and Seed Yield in Soybean

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Abstract : *Photosynthesis and nitrogen metabolism are the most important physiological and biochemical processes in soybean plants. It is important to find agronomical practices to improve these metabolic processes in order to increase grain yield and quality. In this study, a field experiment was conducted using effective microorganisms (EM) to see whether EM can show the expected effects on soybean plants, with three treatments of EM spray concentrations, 0% (CK), 0.1%, and 0.5% at flowering (August 4) and podding (August 14) stages. Metabolic changes of soybean leaves were measured on August 8, August 19, August 29 and September 9 with the 16th, 18th, 18th, 21st functional leaves separately. Photosynthetic rate and stomatal conductance in functional leaves were increased by EM treatments. Nitrate reductase (NR) activities were improved by EM spray treatments. As a consequence, spraying EM increased seed protein and crude fat concentration and increased seed yield.*

Introduction

Foliar applications of plant growth regulators or chemicals are widely used in crop and vegetable cultivation to promote plant growth and enhance yield especially under adverse environmental conditions such as nutrient deficiency and drought. Bio-products and microbial inoculants have been introduced to modern agriculture to produce food with good quality and safety in recent years. A microbial inoculant containing many kinds of naturally occurring beneficial microbes called Effective Microorganisms has been used widely in nature and organic farming (Xu, 2000a). Studies have shown that applying EM increase crop productivity and diseases resistance (Iwaishi, 2000; Mridha et al, 1999; Samy et al, 1995; Sangakara, 1995; Wang et al, 1999; Xu, 2000b; Yamada et al, 2000). However, the mechanism of the effect of EM was not clear in many aspects and there might exist some kinds of active substances in the fermented solution (Xu, 2000b). So far, we know little about whether EM affects seed yield and seed quality in soybean plants. In the present study, photosynthesis, stomatal conductance and nitrate reductase (NR) activity of functional leaves were measured, and seed yield and seed quality were analyzed in field-grown soybean plants with foliar application of diluted EM solutions.

Materials and Methods

Soybean plants (*Gycine max* cv Liaodou 10) were grown in plots each with an area of 5.0x3.0 m^2. The plots were in randomized block design with three replications. Plant density was $1.95x10^4$ h^{-1} with a 0.6-m row spacing and a 0.093-m plant spacing. Organic fertilizer and diammonium phosphate were applied at 15,000 kg h^{-2} and 225 kg h^{-2}, respectively. Foliar applications of EM diluted at 1:1000(0.1% EM) and at 1:500(0.5% EM) were conducted at flowering (August 4) and podding (August 14) stages. The control (C) was spraying water. Photosynthesis and stomatal conductance of

leaves were measured using a LI-6200 Portable Photosynthesis System (LI-COR Inc NE USA) on a clear day during 10:00 - 12:00 am, with gentle wind and a photosynthetic photon fluxes over 1200 μmol m^{-2} s^{-1}. Nitrate reductase (NR) activity in leaves was measured using the live-tissue method.

Results and Discussion

Photosynthesis in functional leaves

Photosynthesis is one of the most important factors that affects soybean seed yield. As shown in Table 1, photosynthesis was much higher in 0.1% EM than in C at the stages of August 19 ($p<0.05$, 18th leaf) and August 29 ($p<0.05$, 18th leaf), and little differences were observed at the stages of August 8 (4days after the first application) and September 10(later growth period). Treatment of 0.5% EM increased photosynthesis significantly at the stages of August 8 (16th leaf) and August 29 (18th leaf). The result suggested that foliar application of EM increased photosynthetic rate in functional leaves during mid-growth period, the key stage when seed yield was determined.

Table 1. Effects of Foliar Applications with EM on Photosynthesis (mmol m^{-2} s^{-1}) in Functional Leaves of Field Grown Soybean Plants

	Date			
Treatment	**August 8**	**August 19**	**August 29**	**September 10**
Water(C)	24.0	19.5	6.80	3.50
0.1% EM	24.2	23.0*	9.90*	4.20
0.5% EM	27.3*	21.0	10.9*	4.00

* Significant at $P<0.05$. The same is for tables below. Leaves measured on August 9, August 19, August 29 and September 10 were the 16th, 18th, 18th and 21st leaf, respectively.

Leaf stomatal conductance

There was close relationship between leaf stomatal conductance and CO_2 assimilation. As shown in Table 2, leaf stomatal conductance was much higher ($p<0.05$) in 0.1% EM and 0.5% EM than in C both during mid-growth period (August 8, August 19, August 29) and during later growth period (September 10). This illustrated that spraying diluted EM solutions on soybean plants weakened stomatal restriction on leaf photosynthesis.

Table 2. Foliar Applications of EM on Leaf Stomatal Conductance (mol m^{-2} s^{-1}) in Field Grown Soybean Plants

	Date			
Treatment	**August 8**	**August 19**	**August 29**	**September 10**
Water (C)	0.955	0.635	0.665	0.291
0.1% EM	1.13*	0.718*	0.698*	0.321*
0.5% EM	1.18*	0.715*	0.704*	0.329*

Leaves measured at different times were the same as in Table 1.

Nitrite reductase activity

Nitrite reductase plays important roles in soybean nitrogen metabolism. As shown in Table 3, NR activity in functional leaves increased significantly in 0.1% EM and 0.5% EM compared with C at the stage of August 8. The activity was much higher in treatment of 0.1% EM than in C at August 19. Little difference was observed among treatments at later growth stage (September 1).

Table 3. Effects of Foliar Applications with EM on NR Activity (μg NO_2-N $g^{-1}FW\ h^{-1}$) in Functional Leaves of Field Grown Soybean Plants

Treatment	Date		
	August 8	August 19	September 1
Water (CK)	114.6	106.5	96.6
0.1% EM	136.1*	129.8*	97.0
0.5% EM	137.1*	107.6	98.0

Measured leaf was 18th

Seed yield and seed quality

Analysis of seed yield and seed quality showed that seed yield increased by 14.3% ($p<0.05$) and 4.5% in 0.1% EM and 0.5% EM than in CK, respectively. The concentrations of protein and crude fat in seed were also higher in treatments of 0.1% EM and 0.5% EM than in C (Table 4).

Table 4. Effects of Foliar Applications with EM on Seed Yield and Seed Quality in Field Grown Soybean Plants

Treatment	Seed Yield (Kg/hm^{-2})	Protein ($g\ kg^{-1}$)	Crude Fat ($g\ kg^{-1}$)
Water (CK)	3398.7	358	199
0.1% EM	3885.7*(+14.3)	378(+5.6)	214(+7.5)
0.5% EM	3552.5(+4.5)	377(+5.3)	212(+6.5)

The value in brackets means increased (+) percentage compared with C.

Conclusions

Soybean plants treated with diluted EM solution (at 0.1% and 0.5%) produced more seed yield with higher protein and crude fat concentration. These were due to the higher nitrate reductase activity and photosynthetic capacity that benefited from higher leaf stomatal conductance in functional leaves during mid-growth period. Results of the present study suggest that EM can be used as a regulation substance to improve metabolisms of crop plants for yield promotion and quality improvement.

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