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Original Research

Improvements in Germination, Growth, and Metabolic Activity of Corn Seedlings by Grain Conditioning and Root Application with *Cyanobacteria* and Microalgae

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Abstract

Latest publications indicate that *Cyanobacteria* and green algae can play an important role in symbiosis with other organisms and can produce active compounds (classified as secondary metabolites) that inhibit the growth of pathogenic bacteria and fungi and increase plant growth. Some strains of *Cyanobacteria* can also assimilate atmospheric nitrogen, which enriches the soil and then is taken up by plants. Due to the lack of information concerning their application in energy crop production, the aim of the presented research was to evaluate the possibility of increasing growth of corn seedlings by grain conditioning and root application with *Cyanobacteria* and green algae. The obtained results show that monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (*Cyanobacteria*), and *Chlorella* sp. (microalgae) significantly increase germination and growth of corn seedlings and also intensify some metabolic processes.

Keywords: corn, *Cyanobacteria*, microalgae, growth, metabolic activity

Introduction

Most activities related to ecological and integrated crop production focus on gaining the high yield of plants that are free of toxic pollution. Results of research conducted in the last few years shed new light on *Cyanobacteria* and microalgae as a potential source of bioactive compounds that can be effectively used in sustainable plant production [1-6]. Application of *Cyanobacteria* as a biofertilizer serves a number of purposes, most importantly the enrichment of the soil and plants with different compounds. There are indications that application of some *Cyanobacteria* strains are able to fix atmospheric nitrogen and enrich the soil with this crucial microelement for plants. This process, as a means of nitrogen fertilization, is being used in rice and wheat cultivation and can be beneficial in ecological agriculture. Additionally, it is thought that *Cyanobacteria* and green algae can produce beneficial growth regulators [7] and active compounds (classified as secondary metabolites) that inhibit the growth of pathogenic bacteria [8-10] and fungi [8, 11, 10] and can increase growth and development of some plant species [4, 5, 6, 12]. Some papers suggest that cyanobacterial activity improves soil structure and porosity by secretion of polysaccharides and mucilage [13]. It has been also determined that they can play an important role in symbiosis with other organisms, including higher plants [14, 15]. In recent years, increasing worldwide interest in the use of *Cyanobacteria* and microalgae in the ecological and integrated production of energy crops, on poor soils, and in adverse conditions of a changing climate has been

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observed. However, the information is very scant in this area, as well as in this, including plant cultivation for consumption purposes.

Due to the lack of information, the aim of the presented research was to investigate the influence of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (*Cyanobacteria*), and *Chlorella* sp. (microalgae) monocultures, suspended in water, on some physiological events and growth of corn seedlings. The used *Cyanobacteria* and microalgae were applied to grains during their conditioning before sowing and to roots via substrate (filter paper) on which grains germinated and then seedlings were grown.

Material and Methods

The commercial grains of corn (*Zea mays* L.) var. Cyrkon, obtained from the breeding company "Nasiona Kobierzyc" in Poland, were used in experiments.

The applied monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (*Cyanobacteria*), and *Chlorella* sp. (microalgae) were cultured at the University of Lódź on BG11 medium (ATCC Medium 616) according to procedure elaborated upon by Romanowska-Duda [16]. Prior to application, each monoculture was filtered and suspended in water. Then the number of cells were counted using a Fuchs-Rosenthal hemocytometer. The cell density used in experiments was estimated to be 2.5×10^5 cells·ml^{-1} water.

Monocultures of *Cyanobacteria* and microalgae suspended in water were applied to corn grains or roots as follows:

1. By moistening grains up to 35% moisture content (m.c.) prior their conditioning (incubation) for 2 days at 20ºC in the tightly sealed flasks, aerated daily. The method of corn grain conditioning using water was elaborated upon in previous research. Grains, after imbibitions in monocultures and conditioning, were dried in laboratory conditions (20ºC, 50% RH) to initial m.c. and then sown on filter papers moistened with distilled water, placed in Phytotoxkit plates, and modified by Romanowska-Duda and Grzesik [17].
2. By continuous moistening of substrate (filter papers) in Phytotoxkit plates modified by authors, on which the unconditioned grains were sown and then the seedlings were grown.

For each treatment 30 replicates (plates) were prepared and every replicate contained 10 grains/seedlings.

The Phytotoxkit plates containing the sown conditioned or not conditioned grains and then the the growing seedlings obtained from them, were kept at 20ºC and 60% RH, under 8 hour dark/16 hour light cycle – SON-T AGRO 400 W, 100 µmol·m^{-2}·s^{-1}. The roots were kept in the dark.

The effects of the grains and filter paper moistening with the mentioned monocultures of *Cyanobacteria* and microalgae, on germination and seedling development, was evaluated on the base of measurements of a number of the germinated grains, dynamics of germination, mean germination time and measurements of the roots and leaf lengths, fresh and dry weight (g) of roots and leaves of seedlings, index of chlorophyll content, activity of net photosynthesis (µm CO_2·m^{-2}·s^{-1}), transpiration (mmol H_2O·m^{-2}·s^{-1}), stomatal conductance (mmol H_2O^{-1}·m^{-2}·s^{-1}), intercellular CO_2 concentration (µmol CO_2 air·mol^{-1}), activity of acid (pH=6.0) and alkaline phosphatase (pH=7.5) (mU·g^{-1} f.w.), RNase (mU·g^{-1} f.w.), total dehydrogenase (mg·g leaves^{-1}), and electrolyte leakage from leaves (µS·g^{-1}·ml^{-1}).

Germination of grains was evaluated at 20ºC. They were counted as germinated when the radicals protruded through the grain coat. Germination (radical protrusion) was scored on a daily basis and grain germination percentage and mean germination time (MGT) were calculated using Seed Calculator Version 3.0, a computer program developed by Plant Research International B.V., Wageningen, The Netherlands [8].

Lengths of roots and leaves were measured in two-day intervals with ruler, from grain to the end portions [18]. Fresh and dry weights of seedlings were evaluated at the end of experiments. Dry weight of seedlings was measured after 3 days drying at 130ºC.

Index of chlorophyll content in leaves was evaluated using a Minolta SPAD-502 chlorophyll meter (Konica Minolta) and expressed in SPAD units [19].

Activity of photosynthesis, exhibited by the net photosynthesis, stomatal conductance, intercellular CO_2 concentration, and transpiration, was measured in the infrared light using the gas analyzer apparatus TPS-2, PP Systems (USA) [20].

Activity of acid and alkaline phosphatase and RNase in leaves was examined according to methods described by Knypl and Kabzinska [21].

Electrolyte leakage was investigated at 20ºC after placing leaf segments in test-tubes and adding 3 ml of distilled water. Electrolyte leakage was measured after 2 and 4 hours using a CC-551 Elmetron microcomputer conductivity meter [18].

Results concerning germination, seedling growth, index of chlorophyll content, activity of photosynthesis, activity of acid and alkaline phosphatase and RNase, as well as electrolyte leakage from leaves were analyzed using analysis of variance. The means were separated using Duncan's multiple range test (LSD) at an alpha level of 0.05.

Results and Discussion

The obtained results showed that monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (*Cyanobacteria*), and *Chlorella* sp. (microalgae), suspended in water and applied to the conditioned corn grains or added to roots via substrate with growing seedlings greatly increased germination, growth of seedlings, their fresh and dry weight, and metabolic activity (Figs. 1, 2 Tables 1-3). However, the improvements in germination and seedling development depended on the methods of applications and slightly on species of *Cyanobacteria* or microalgae used. The obtained results are in line with our other studies, showing positive influ-

Table 1. Effects of *Cyanobacteria* and microalgae application on the conditioned grains and substrate (filter paper on which grains were sown and seedlings grown) on germination and corn plant growth.

Method of *Cyanobacteria* or microalgae application	Cyanobacteria and microalgae applied to seeds or roots					$LSD_{0.05}$
	Control	H_2O	*Microcystis aeruginosa* MKR 0105	*Chlorella* sp.	*Anabaena* PCC 7120	
Number of germinated grains (%)						
Grain cond.	90.7 a*	95.1 b	100 c	100 c	100 c	4.34
Appl. to substr.		-	100 c	100 c	100 c	
Mean time of germination (days)						
Grain cond.	4.49 e	3.59 d	2.72 a	3.03 b	2.83 b	0.15
Appl. to substr.		-	3.02 b	3.33 c	3.19 c	
Length of roots after 8 days from grain sowing (cm)						
Grain cond.	5.6 a	6.4 b	10.3 f	9.8 e	8.7 d	0.40
Appl. to substr.		-	8.7 d	7.7 c	7.5 c	
Length of leaves after 14 days from grain sowing (cm)						
Grain cond.	17.6 a	19.9 b	26.7 e	25.5 d	22.4 c	1.11
Appl. to substr.		-	21.7 c	20.2 b	20.2 b	
Fresh weight of roots after 14 days from grain sowing (g)						
Grain cond.	4.3 a	6.0 b	8.9 f	8.3 e	8.1 d	0.20
Appl.to substr.		-	8.0 d	7.5 c	7.4 c	
Dry weight of roots after 14 days from grain sowing (g)						
Grain cond.	1.0 a	1.2 b	1.9 g	1.8 f	1.6 e	0.09
Appl. to substr.		-	1.6 e	1.5 d	1.4 c	
Fresh weight of leaves after 14 days from grain sowing (g)						
Grain cond.	3.3 a	3.7 b	5.0 f	4.8 e	4.6 d	0.15
Appl. to substr.		-	4.6 d	4.4 c	4.3 c	
Dry weight of leaves after 14 days from grain sowing (g)						
Grain cond.	0.4 a	0.5 b	0.9 f	0.8 e	0.7 d	0.09
Appl. to substr.		-	0.8 e	0.7 d	0.6 c	

*The data marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05.

ence of cyanobacterial cell suspensions on grape development and sunflower seed germination and plant growth. They also shoved dependence between growth of particular species plant, cyanobacterial strain, and seed conditioning [5].

In the presented research applications of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120, and *Chlorella* sp. monocultures to the conditioned corn grains and roots significantly increased the number of the germinated grains, dynamics, and mean time of germination and accelerated growth of seedlings, exhibited by faster elongation of roots and leaves and enlarged their fresh and dry biomass. These events were associated with the increased index of chlorophyll content in leaves, activity of net photosynthesis, transpiration, stomatal conductance, intercellular CO_2 concentration, activity of acid (pH=6.0) and alkaline phosphatase (pH=7.5), RNase, total dehydrogenase, and decreased electrolyte leakage from leaves (Figs. 1-4 Tables 2, 3). Reduced electrolyte leakage from the leaves indicates the lower permeability of cytomembranes under the influence of application of the investigated *Cyanobacteria* and microalgae. The presented research and also these performed on rice and wheat indicate that the high concentration of different bioactive compounds included in microalgae and *Cyanobacteria* and the possibility of them to assimilate atmospheric nitrogen, makes these organisms very useful in stimulating plant growth [1-7, 22-24].

The evaluation of effectiveness of the used particular monocultures show that *Microcystis aeruginosa* MKR

0105 was slightly more profitable in increasing seedling growth than *Anabaena* sp. PCC 7120 and *Chlorella* sp. (Figs. 1-3, Tables 1-3).

The presented research shows that the increased metabolic activity, accelerated corn grain germination, and faster seedling development were associated also with their higher health status, as it was also observed in the other experiments showing inhibition of pathogenic bacteria growth [8-10] and fungi [8, 11, 10].

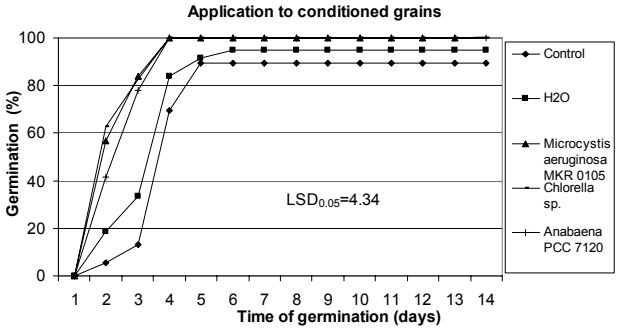

Fig. 1. Dynamics of corn grain germination as affected by the pre-sowing application of monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (*Cyanobacteria*), and *Chlorella* sp. (microalgae) to the conditioned grains instead of water.

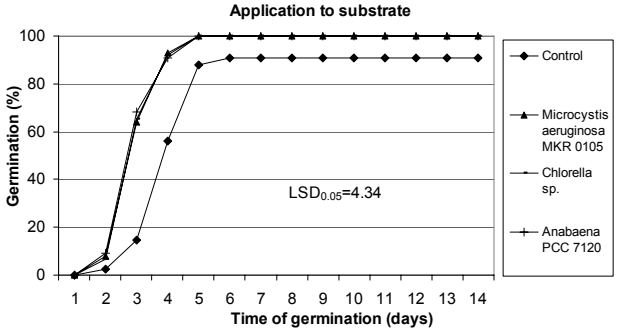

Fig. 2. Dynamics of corn grain germination as affected by the application of monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (Cyanobacteria), and *Chlorella* sp. (microalgae) to substrate (filter papers) on which the grains were sown and seedlings grown.

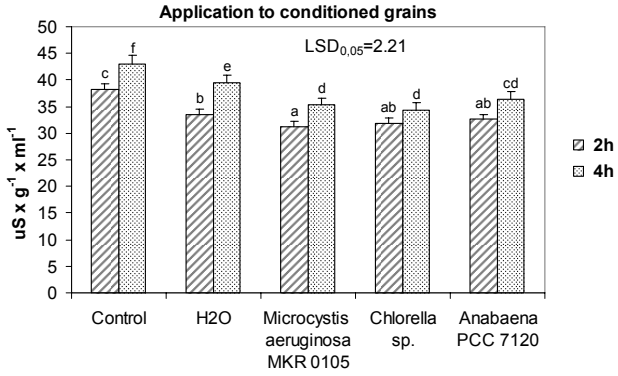

Fig. 3. Electrolyte leakage from corn leaves, placed in water for 2 and 4 hours, as affected by the pre-sowing conditioning of grains in H_2O and in monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (Cyanobacteria), and *Chlorella* sp. (microalgae).
The data marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05.

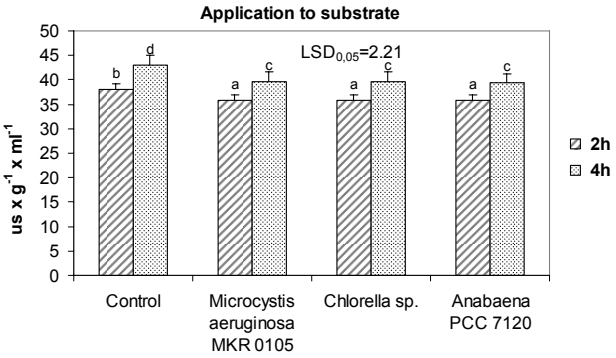

Fig. 4. Electrolyte leakage from corn leaves, placed in water for 2 and 4 hours, as affected by the application of monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 (Cyanobacteria), and *Chlorella* sp. (microalgae) to substrate (filter papers) on which the grains were sown and seedlings grown.
The data marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05.

The obtained results show that the used monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120, and *Chlorella* sp. increased germination, seedling growth, and their metabolic processes independently of application methods. Although applying them to grains during conditioning, before sowing, was more profitable than the continuous application to roots via substrate (filter paper) on which the seedlings grew (Figs. 1, 2, Table 1). Conditioning included imbibitions of grains in water or in the mentioned monocultures under controlled conditions and incubation, to initiate the metabolic pre-germination processes, but preventing the penetration of the grain coat by embryonic root. Thus, conditioned grains germinated immediately after sowing on wet substrate, while in the unconditioned seeds the metabolic processes were initiated only after sowing, and thus resulted in delayed germination [24-26]. In the presented research conditioning of corn grains in water caused earlier germination and faster seedling growth, as compared to control. Application of monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120, and *Chlorella* sp. to corn grains before their incubation accelerated additional initiation and course of the pre-germination metabolic processes. This resulted in more accelerated germination and increased seedling development as compared to effects of conditioning in water. Otherwise, application of the used monocultures of *Cyanobacteria* and microalgae to substrate (filter paper) also caused accelerated germination and seedling growth compared to water treatment. However, they were delayed (in comparison to the conditioned grains in the mentioned monocultures) due to the initiation of pre-germination processes only after grain sowing (Figs. 1, 2. Table 1) [12, 25, 26].

The obtained results, as well as the previous literature data [1-6], indicate that the application of various forms of

Table 2. Effects of *Cyanobacteria* and microalgae application on the conditioned grains and substrate (filter paper on which grain were sown and seedlings grow) on the photosynthesis activity and index of chlorophyll content in corn leaves.

Method of *Cyanobacteria* or microalgae application	Cyanobacteria and microalgae applied to grains or roots					$LSD_{0.05}$
	Control	H_2O	*Microcystis aeruginosa* MKR 0105	*Chlorella* sp.	*Anabaena* PCC 7120	
Nett photosynthesis (μm $CO_2 \cdot m^{-2} \cdot s^{-1}$)						
Grain cond.	4.3 a*	4.7 b	5.6 d	5.5 d	5.1 c	0.24
Appl. to substr.		-	5.1 c	5.0 c	5.0 c	
Transpiration (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)						
Grain cond.	0.76 a	0.89 b	1.24 d	1.22 d	1.21 d	0.04
Appl. to substr.		-	1.17 cd	1.14 c	1.13 c	
Stomatal conductance (mmol $H_2O^{-1} \cdot m^{-2} \cdot s^{-1}$)						
Grain cond.	58 a	76	101 c	100 c	101 c	3.1
Appl. to substr.		-	97 b	97 b	95 b	
Intercellular CO_2 concentration (μmol CO_2 air$\cdot mol^{-1}$)						
Grain cond.	420 e	417 cd	402 a	407 b	406 b	2.3
Appl. to substr.		-	411 c	413 c	415c	
Index of chlorophyll content in leaves						
Grain cond.	28.1 a	29.9 b	37.2 e	35.4 d	32.8 c	1.59
Appl. to substr.		-	35.1 d	34.5 d	31.6 c	

*The data marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05.

Cyanobacteria and microalgae, including *Microcistis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120, and *Chlorella* sp., can be beneficial to growth, development, and metabolic activity of corn seedlings. Some of them have the remarkable ability to form intimate symbiotic associations with a wide range of eukaryotic hosts belonging to different plant groups. *Cyanobacteria* also are a biogeochemically important component of diverse ecosystems that play a significant role in carbon and nitrogen cycling. Non-toxic cyanobacterial (without microcystins; MC) and microalgae cultures can be used for ecological and integrated corn cultivation and will facilitate environmental protection by reducing the need to use toxic artificial fertilizers. Other benefits from the presence of non-toxic *Cyanobacteria*, like as *Anabaena* sp. and *Calothrix* sp., is the stimulated production of ammonia and indolic compound. These strains also inhibited pathogenic fungi (*M. phaseolina*) [27, 28]. El Modafara et al. [29] demonstrated the effects of treatments of tomato seedlings with bioelicitors obtained from green algae (*U. lactuca*) and contained polysaccharides (glucuronan, ulvan) in the cell-walls. These elicitors have properties of natural defenses, accompanied by a systemic acquired resistance that seems to be salicylic acid-dependent and can be useful for crop protection. Elicitors, compared to that of other algae polysaccharides (carrageenan, laminarin and alginate), significantly reduced wilt development caused by *Fusarium oxysporum* f. sp. *Lycopersici*. Swarnalakshmi [30] presented an investigation toward evaluating novel biofilmed preparations, using *Cyanobacteria* (*Anabaena torulosa*) as a matrix for agriculturally useful bacteria (*Azotobacter*, *Mesorhizobium*, *Serratia,* and *Pseudomonas*) in wheat crop. The performance of such phototrophe-heterotroph biofilmed preparations was evaluated using individual cyanobacterium, available bacterial inoculants, and dual cultures of the partners. These results showed that the interrelationships of nitrogen fixation with increased P uptake by plant exist. This synergism among the partners, emphasizes the need for evaluation at field level for their promise as a green technology for agriculture.

However, due to the limited amount of literature data and the increasing worldwide interest in this area, further studies are required for a more complete understanding of this phenomenon and confirmation of the above results, as well as identification of active compounds released by *Cyanobacteria* and microalgae strains.

Conclusions

1. New strategies of crop protection are needed urgently to minimize the chemical quantities of pesticides in the soil and their residues in food products.
2. The deployment of non-toxic monoculture of *Cyanobacteria* and green algae as biofertilizer input for increasing soil nutrient availability reduces the dependence on costly chemical fertilizers in integrated nutrient management system.

Table 3. Effects of *Cyanobacteria* and microalgae application on conditioned grains and substrate (filter paper on which grain were sown and seedlings grow) on activity of selected enzymes in corn leaves.

Method of *Cyanobacteria* or microalgae application	Cyanobacteria and microalgae applied to grains or roots					$LSD_{0.05}$
	Control	H_2O	*Microcystis aeruginosa* MKR 0105	*Chlorella* sp.	*Anabaena* PCC 7120	
Activity of acid phosphatase (pH=6.0) (mU·g^{-1} f.w.)						
Grain cond.	0.60 a*	0.75 b	0.92 e	0.85 cd	0.86 d	0.05
Appl. to substr.		-	0.86 d	0.79 b	0.80 bc	
Activity of alkaline phosphatase (pH=7.5) (mU·g^{-1} f.w.)						
Grain cond.	0.20 a	0.30 b	0.50 f	0.45 cd	0.48 ef	0.02
Appl. to substr.		-	0.45 de	0.43 c	0.45 cd	
Activity of RNase (mU·g^{-1} f.w.)						
Grain cond.	3.05 a	3.51 b	4.52 f	4.10 d	4.19 e	0.18
Appl. to substr.		-	4.30 e	3.50 bc	3.60 c	
Total dehydrogenase activity (mg·g leaves^{-1})						
Grain cond.	0.51 a	0.60 b	0.78 g	0.75 f	0.66 d	0.02
Appl. to substr.		-	0.70 e	0.68 de	0.63 c	

*The data marked with the same letters are not significantly different, according to Duncan multiple range test at an alpha level of 0.05.

3. Application of various forms of *Cyanobacteria* and microalgae (*Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120, and *Chlorella* sp.) can be beneficial to growth, development, and metabolic activity of corn plants.
4. Applying cyanobacterial and microalgae monocultures to conditioned grains is more profitable than continuous application to roots via substrate (filter paper) on which the seedlings grow.
5. Non-toxic cyanobacterial and microalgae cultures can be used for ecological and integrated corn cultivation and will facilitate environmental protection by reducing the need to use toxic artificial fertilizers.
6. New biological approaches and the stimulation of natural plant defense is considered one of the most promising alternative strategies for crop protection.
7. Cell suspension of prokaryotic or eukaryotic cells can play a key role in plant-microbe interactions.

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