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RESEARCH ARTICLE

Interactive effect of simulated acid rain, calcium silicate, and y-aminobutyric acid on physiological processes in corn and wheat

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Abstract

The effect of calcium silicate, y-aminobutyric acid (GABA), and their mixture on the adaptation of wheat and corn to simulated acid rain has been evaluated in the pot experiments. Acid rain was simulated by watering twice with distilled water acidified with sulfuric acid to pH = 2. Test plants were grown in a plant growth chamber under controlled conditions of temperature, illumination and relative humidity for 18 days. The physiological state of the test plants was assessed by characteristics of growth (shoot height, root length, dry weight of shoots and roots), the content of photosynthetic pigments, flavonoids, and proline in leaves. For the corn, the content of anthocyanins in shoots and roots was also evaluated. In parallel, the physical and chemical characteristics of the soil (pH, electrical conductivity, redox potential, content of soluble carbonates, and nitrates) were determined.

It was established that simulated acid rain inhibited the growth and accumulation of photosynthetic pigments in the leaves of wheat and corn. The content of protective metabolites (proline, flavonoids, and anthocyanins) increased. Wheat showed greater sensitivity to the inhibiting effect of acidification compared to corn. The application of $CaSiO_3$ was more effective than GABA in restoring pH value and HCO_3^- concentration in soil, while the application of GABA more effectively promoted the accumulation of NO_3^- anions in soil. Combining $CaSiO_3$ with GABA was the most effective in restoring soil physical and chemical properties altered by simulated acidification and stimulating the growth and photosynthesis in the test-plants. Thus, the mixture of $CaSiO_3$ with GABA is promising for further studies of the possibility of its application to mitigate the negative impact of acid depositions on vegetation and soil.

Keywords: acid rain, calcium silicate, γ-aminobutyric acid, corn, wheat, growth, photosynthetic pigments, proline, flavonoids, anthocyanins

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Introduction

One of the dangerous consequences of anthropogenic emissions of sulfur and nitrogen compounds into the atmosphere is an increase in the acidity of precipitations. This problem has been relevant to the world for over 50 years. Every year, 17 million tons of harmful substances are released into the atmosphere throughout Ukraine, about 70% of which are products of incomplete fuel combustion in thermal power plants and vehicles, which pose a significant threat in terms of acid precipitations (Nichuk, 2020). Acid deposits caused the greatest damage to vegetation. In particular, acid rain may cause visible leaf damage, anatomical alterations of foliar tissues as well as intervene with the basic physiological processes such as photosynthesis, respiration, mineral nutrition, water balance, etc. (Debnath et al., 2018; Debnath & Ahammed, 2020; Rodríguez-Sánchez et al., 2020).

Acid depositions decrease the pH of the soil, which promotes the leaching of minerals (especially calcium, potassium, and magnesium), inhibits microbiological activity in the root layer, and contributes to the accumulation of phytotoxic concentrations of aluminum, iron, and manganese. Acidification of the soil environment makes plants more susceptible to diseases and destructive effects caused by radionuclides, heavy metals, etc. (Kovalchuk 2004; Nichuk, 2020). Acid depositions are thought to be the main cause of the weakening of the viability of the wood stands and the spread of new diseases of trees, which are presently observed in many regions of the world (Kovalchuk, 2004; Battles et al., 2014).

A growing number of researchers have their efforts committed to elaborate approaches to reduce the negative consequences of acid depositions to vegetation. Most of them have been focused on the recovery of acidified soil (Battles et al., 2014; Fowler et al., 2022), and only a small amount of studies consider the physiological adaptation of higher plants (Liu et al., 2018).

Traditional approaches to control soil acidification are based on applying lime, lime rock, or other Ca-containing minerals. Good prospects for lime application to compensate the negative consequences

of acidic depositions in the Monongahela National Forest in West Virginia were shown by Fowler et al. (2022). In this study, liming with a helicopter a total of 323 ha at the rate of 10 Mg ha⁻¹ reduced acidity values by 73%, Al bioavailability - by 80%, and increased Ca concentrations three-fold in O and A horizons (Fowler et al., 2022). In another study, experimental amendment of acidified soil with wollastonite (CaSiO₂) in Hubbard Brook Experimental Forest (New Hampshire, Canada) was shown to compensate for the negative effect of acid deposition on tree biomass increment, promoted higher aboveground net primary production, and increased the leaf area index (Battles et al., 2014). Our previous studies showed that natural siliceous minerals mixed with organic fertilizers alleviated soil acidity stress in wheat and corn in the model pot experiments as well as in the field trials (Zaimenko et al., 2015, 2016). In particular, all the tested amendments compensated the negative impact of acidification on the contents of the photosynthetic pigments in leaves, the growth of shoots and roots of wheat and corn, and optimized the course of redox processes that increase the pH (especially the mixtures based on potassium silicate and peat), reduced the electrical conductivity and phytotoxicity of the soil (Zaimenko et al., 2015, 2016).

Another approach is based on applying biostimulants to enhance higher plants' adaptive responses to environmental stresses connected with acid deposition (Liu et al., 2018). Inoculation of higher plants with mycorrhizal fungi or symbiotic nitrogen-fixing rhizobia is known to have the potential to raise their tolerance to a range of environmental stresses, including acidification (Msimbira & Smith, 2020). Though molecular mechanisms of such protection are not fully understood until now, better nutrient uptake and increased production of phytohormones resulted from microbial inoculants are thought to be among the important contributors to these phenomena. In particular, IAA and organic acids produced by phosphate-solubilizing bacteria of Burkholderia thailandensis. B. seminalis, and Sphingomonas pituitosa were shown to improve rice root growth and seedlings development under acidity stress, which indicated the potential of these isolates to be used in a bio-fertilizer formulation for rice cultivation on acid sulfate soils (Panhwar et al., 2014). Exogenous plant growth regulators, such as kinetin, 6-benzylaminopurine, were shown to protect crops against adverse effects of environmental stresses, including soil acidity (Čižková, 1992; Gadallah, 1994).

The four-carbon amino acid gammaaminobutyric acid (GABA) has been shown to control many physiological responses during environmental stresses in higher plants (Wang et al., 2021). Exogenous GABA application was shown to improve tolerance to heat, chilling, drought, phytopathogens, insects, Al-toxicity, hypoxia, salinity, and allelopathic stress in some crops (Didyk, 2017; Ramos-Ruiz et al., 2019; Wang et al., 2021; Zhou et al., 2021). The protective effect of exogenous GABA is explained by its participation in regulating the tricarboxylic acid cycle, nitrogen reservoir, cytoplasmic pH, antioxidant defense, and osmotic potential (Ramos-Ruiz et al., 2019; Wang et al., 2021; Zhou et al., 2021). Although GABA has been proven beneficial for plants adaption to abiotic stresses, there is a very limited knowledge about its interaction with stress caused by acid depositions and its interactions with other stress protectants such as biologically active silicon, etc.

The objective of our study was to evaluate the effect of calcium silicate and GABA separately and as a mixture on the adaptation of wheat and corn to simulated acid deposition. At the same time, it was planned to determine the modifying role of soil in limiting the negative impact of acidification on the environment.

Material and methods

Test plants, experimental setup, and cultivation conditions

Pot experiments simulating acid rain were conducted at the department of allelopathy of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine. The seeds of the test plants of wheat (*Triticum aestivum* L. 'Smuglyanka') and fodder corn (*Zea mais* L. 'Kadr 267 MV') were sown in pots (eight seeds per pot with a volume of 300 ml) filled with gray podzolic soil, which had been dried, sieved through a 2 mm sieve and sterilized in the oven at 100 °C beforehand. Acid precipitations were simulated by watering twice with distilled

water acidified with sulfuric acid to the pH=2 (40 ml per pot): the day and three days after sowing seeds of the test plants. Instead of acid precipitation, distilled water (pH=7) was used in control. The experiments included the following treatments: without acid precipitations and any soil amendments (Control); simulated acid precipitations but without any soil amendments (SAR); simulated acid precipitations and CaSiO₂ applied at a rate of 0.1% to the dry weight of the soil (SAR, CaSiO₃); simulated acid precipitations and GABA applied at a rate of 0.03% to the dry weight of the soil (SAR, GABA); simulated acid precipitations and the mixture of CaSiO₂ and GABA (0.1% + 0.3%) to the dry weight of the soil) (SAR, $CaSiO_3 + GABA$).

The test plants were grown in a plant growth chamber at a temperature of 24–26 °C, 14/10 h (light/dark), light at 80 μ mol photons m^{-2} s⁻¹, and soil moisture of 60–75% of the full physical water holding capacity. The duration of the experiments was 18 days for wheat and 22 days for corn. At the end of the experiments, test-plants were dug up, and their roots were rinsed with tap water and blotted up with filter paper. The replication of experiments was fourfold. The physiological state of wheat and corn plants was assessed by morphometric characteristics of growth (shoot height, root length, dry weight of shoots and roots), the content of photosynthetic pigments, flavonoids, and proline in leaves. The content of anthocyanins in shoots and roots was also evaluated for corn.

Measurements

Morphometric and biochemical measurements were conducted on the 18th day of cultivation for wheat and the 22nd day - for corn. Photosynthetic pigments (chlorophylls *a* and *b*, and carotenoids) were extracted from freshly collected leaves with dimethylsulfoxide (Hiscox & Israelstam, 1979). Quantitative content was determined using a spectrophotometer SPECORD 200 (Analytik Jena), according to Wellburn (1994). Flavonoids were extracted with 70% ethanol. Quantitative analysis was performed using the spectrophotometer SPECORD 200 (Analytik Jena) after а qualitative reaction with aluminum chloride diluted in 95% ethanol to a concentration of 2% (Komarova et al., 1998). Anthocyanins were

Treatment	Shoot height, mm	Root length, mm	Adventitious roots number	Shoot dry weight, mg	Root dry weight, mg
Wheat					
Control, pH=7	287	147	3.1	28.7	6.4
SAR, pH=2	226	125	2.9	22.2	4.1
SAR, $CaSiO_3$	282	141	3.9	25.9	6.8
SAR, GABA	289	154	4.1	29.5	10.0
SAR, CaSiO ₃ +GABA	302	173	3.5	31.3	11.5
LSD	5.6	4.4	0.1	2.2	3.0
Corn					
Control, pH=7	298	193	4,3	112,5	42,5
SAR, pH=2	238	149	4.6	90.6	38.9
SAR, CaSiO ₃	276	178	5.6	81.2	32.6
SAR, GABA	294	187	5.2	92.5	33.4
SAR, CaSiO ₃ +GABA	295	194	5.6	110.4	45.2
LSD	4.9	2.8	0.2	1.8	2.4

Table 1. Morphometric characteristics of wheat and corn exposed to simulated acid precipitation with and without soil amendments.

Note. SAR – simulated acid rain, LSD – least significant difference at P < 0.05.

extracted from freshly harvested shoots and roots of corn with 0.1 N hydrochloric acid. Quantitative analysis was performed using the spectrophotometer SPECORD 200 (Analytik Jena) according to the method (Pisarev et al., 2010).

The pH of the soil solution was measured at the end of the experiment with a Cond 315i conductometer (WTW GmbH, 2015). The redox potential was determined using a pH/ ORP Meter HI 2211 (Hanna Instruments, 2005). Preparation of soil samples for analysis was performed according to Rinkis-Nollendorff (Rinkis & Nollendorff, 1982). The content of soluble carbonates in the soil solution was determined by titration with sulfuric acid with the addition of the methyl orange indicator until the color of the solution changed from yellow to orange (Pecheneva, 1998). The content of nitrates was determined spectrophotometrically using a qualitative diphenylamine (Rinkis reaction with & Nollendorff, 1982).

Statistical analysis

Statistical processing of the results of the experiments was carried out by the method of

ANOVA with the help of Statistica 10.0 software (Stat Soft. Inc., Tulsa, USA, 2011). P values of less than 0.05 were considered statistically significant.

Results and discussion

In our studies, the simulated acid rain inhibited the growth of shoots and roots and the accumulation of photosynthetic pigments in the leaves of wheat and corn. At the same time, the content of protective metabolites (proline, flavonoids, and anthocyanins), which are stress indicators, increased in shoots and roots (Tables 1 & 2; Figs. 1 & 2). Wheat showed greater sensitivity to the inhibiting effect of simulated acidification compared to corn. In particular, the length of shoots and roots of the wheat seedlings was inhibited by 21% and 16%, respectively. In corn seedlings, the inhibition of shoot height reached 11% and was insignificant for root growth. The content of chlorophyll a in the leaves of wheat seedlings was inhibited by 15% under simulated acid rain. While in corn, the corresponding inhibition value was not significantly different from the control. As observed in our studies,



Figure 1. Effect of simulated acid rain and soil amendments on the growth of wheat (A) and corn (B) test-plants in pot experiments: control (1); simulated acid rain without any amendments (2), with the application of $CaSiO_3$ (3), with the application of GABA (4), and with the application of $CaSiO_3$ and GABA mixture (5).

Treatment	Photosynthetic pigments				Dualia
	Chlorophyll a	Chlorophyll b	Carotenoids	— Flavonoids	Proline
Wheat					
Control, pH=7	8.4	2.9	1.6	0.38	0.1
SAR, pH=2	7.1	2.4	1.2	0.53	0.2
SAR, CaSiO ₃	8.2	2.7	1.6	0.38	0.15
SAR, GABA	9.2	3.1	1.	0.36	0.18
SAR, CaSiO ₃ +GABA	9.5	3.3	1.9	0.36	0.18
LSD	0.93	0.61	0.22	0.04	0.02
Corn					
Control, pH=7	14.4	6.8	2	0.42	0.05
SAR, pH=2	11.8	5.5	1.6	0.62	0.22
SAR, CaSiO ₃	14.8	7.2	2.2	0.41	0.1
SAR, GABA	14.2	6.7	2	0.46	0.13
SAR, CaSiO ₃ +GABA	14.9	7.4	2.4	0.44	0.15
LSD	0.82	0.44	0.24	0.03	0.04

Table 2. The content of photosynthetic pigments, flavonoids, anthocyanins, and proline (mg/g of fresh plant weight) in the leaves of wheat and corn exposed to simulated acid precipitation with and without soil amendments.

Note. SAR – simulated acid rain, **LSD** – least significant difference at P < 0.05.

the higher tolerance of corn to simulated acid rain could be explained by the better capacity of its antioxidant defense system to respond to acidification than wheat. In particular, the proline content in the corn leaves subjected to simulated acid rain was 4.4-fold higher compared to the control. While in wheat, the increase of proline content was only 2-fold. The same tendency was observed for flavonoids, the total content of which increased by 47% in corn, and only by 12% in wheat under simulated acidification. The content of anthocyanins in the corn shoots and roots increased by 54% and 72%, respectively, under simulated acid rain, indicating an important role of these antioxidants in the adaptation of corn to acid depositions.

Analysis of the soil's physical and chemical characteristics showed that soil pH was restored to the initial level until the end of the experiments when the corn was used as a test plant, while in wheat, it was somewhat lower but still within optimum values (Table 3). This testifies to the good buffering properties of the soil used in our study. It is known that the mechanical and chemical composition of soils significantly affects its resilience to the influence of acid precipitation (Ma et al., 2020). In particular, Kovalchuk (2004) studied the effect of simulated acid rain with a pH of 2.5 for three years on acidity and the content of macro- and microelements in soils of different mechanical compositions and established that sandy soils were the most vulnerable. By the end of the experiments, the decrease in the pH of water in sandy substrates was from 0.2 to 0.8 units, and that of saline – was from 0.4 to 1.4. Similar trends are described in the study of Wei et al. (2020).

soil Other characteristics, such as conductivity and the content of NO_3^- and HCO₃⁻ anions, were shifted significantly due to simulated acid rain: conductivity demonstrated the tendency to increase, while the content of NO_3^- and HCO_3^- anions decreased. The negative influence of simulated acid rain on the amount of bioavailable nitrogen was demonstrated in other studies (Cho et al., 2002; Ma et al., 2020). This tendency was explained by increased N mobility as well as inhibition of nitrification and nitrogen fixation process in soil (Li et al., 2019). The decrease in carbonate content observed in our experiments could also be caused by a decline in the intensity of

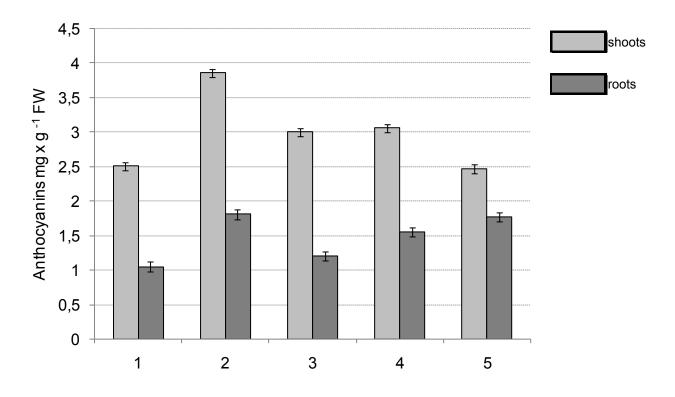


Figure 2. Content of anthocyanins in shoots and roots of corn in control (1) and under simulated acid rain without any amendments (2), with the application of $CaSiO_3$ (3), with the application of GABA (4), and with the application of $CaSiO_3 + GABA$ mixture (5) to the soil substrate. Vertical bars are the least significant difference at P < 0.05.

mineralization processes in the soil. Li et al. (2019) also showed a reduction in the content of mineral carbon and an increase in organic carbon in forest soils under the influence of simulated acid rain.

Application of CaSiO₃ was more effective than GABA in restoring pH value and concentration of HCO₃⁻ anions in soil. The application of GABA was more effective in promoting the accumulation of NO₃⁻ anions in soil solution. Combining CaSiO₃ with GABA enabled reaching the highest levels of both mentioned anions and restoring the initial pH level and the redox potential. The concentration of HCO_3^- reached the control level (pH=7) in the case of adding a mixture of CaSiO₃ and GABA indicates the restoration of the carbonate-calcium system $CaCO_3$ -Ca(HCO_3)_2-CO, which is one of the mechanisms of soil buffering. Thus, applying CaSiO₂, GABA, and their mixture stimulates the adaptation of test plants to acid stress due to the effect on the buffer properties of the soil and physiological processes in the test plants.

Application of CaSiO₃ partially compensated the negative effect of simulated acidification on

the test-plants' morphometric and biochemical characteristics, such as chlorophyll content, shoot height, and root length, but stimulated adventitious root formation. In contrast, the content of defensive antioxidants (proline, anthocyanins) flavonoids. was reduced compared to the plants growing without any soil amendments. However, these biochemical characteristics remain higher than in the control (without simulated acid rain). The application of GABA and its mixtures with CaSiO₃ completely compensated for the negative effect of simulated acid rain on the accumulation of photosynthetic pigments in leaves and the growth of the test-plants. In the case of applying the mixture to the soil before sowing the seeds of test plants, the growth rates of shoots and roots, as well as the content of chlorophyll a significantly exceeded the corresponding rates of plants growing in control. The flavonoid content was lower than in the untreated plants under acid stress conditions but higher than in the control and the variant treated with CaSiO₃ alone.

In a wide range of studies on various crops such as lentils, melon, rice, wheat, and corn, exogenous GABA was shown to effectively

Treatment	рН	Conductivity, µS∕cm	NO ₃ , ppm	HCO ₃ , mol/l	Redox potential, mV
Wheat					
Control, pH=7	7.04	111	30	1.6	138
SAR, pH=2	6.95	131	16	1.3	135
SAR, $CaSiO_3$	7.02	187	22	1.5	133
SAR, GABA	7.01	201	46	1.4	134
SAR, CaSiO ₃ +GABA	7.02	174	48	1.6	138
LSD	0.02	1.87	1.55	0.07	1.71
Corn					
Control, pH=7	7.02	68	15	1.5	119
SAR, pH=2	7.02	84	13	1.1	118
SAR, $CaSiO_3$	7.04	100	14	1.4	121
SAR, GABA	7.01	93	15	1.4	123
SAR, CaSiO ₃ +GABA	7.02	76	15	1.5	121
LSD	0.03	1.22	2.47	0.04	1.93

Table 3. Soil physical and chemical characteristics after cultivation of wheat and corn exposed to simulated acid precipitation with and without soil amendments.

Note. SAR – simulated acid rain, **LSD** – least significant difference at P < 0.05.

alleviate inhibition of germination and growth processes under unfavorable environmental conditions such as extreme temperatures, drought, water, salt, light or hypoxia (Ramos-Ruiz et al., 2019). Some authors relate the stress-protective effect of GABA with its stimulation of photosynthetic activity and antioxidant defense systems (Ramos-Ruiz et al., 2019). Without environmental stress, the exogenous GABA is known to affect growth and morphogenesis in higher plants. The study of Li et al. (2016) demonstrated that corn seedlings exposed to exogenic GABA significantly increased root and shoot fresh weights, net photosynthesis rate, chlorophyll content, the activity of antioxidant enzymes, and enzymes of the nitrogen metabolism (Ramos-Ruiz et al., 2019).

The impact of acid deposition on vegetation is a complex phenomenon involving various processes, including changes in the soil environment, associated microbiota, and direct effects on higher plants' physiological performance. Therefore, ecosystem-based approaches should be applied to protect crops against acid deposition. In this respect, restoration of soil buffer capacity and balance of nutrients precondition normal development

of soil microbiota and crops health. In our study mixture of $CaSiO_3$ and GABA was the most promising in terms of restoration of the soil environment as well as the physiological performance of the tested crops under simulated acid rain.

Conclusions

In summary, this study demonstrated good prospects for applying Ca-containing minerals in combination with the growth regulators (including GABA) to enhance wheat and corn resistance to environmental stresses connected with acid deposition. Both components of the mixture complemented each other in restoring the tested physical and chemical properties of the soil altered by simulated acidification and stimulated test-plants' growth and photosynthesis. The obtained results confirmed the involvement of low molecular weight antioxidants, such as proline, flavonoids, and anthocyanins, in adapting the studied cereals to simulated acidification.

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Сумісна дія імітованого кислотного дощу, силікату кальцію та у-аміномасляної кислоти на фізіологічні процеси в рослинах пшениці та кукурудзи

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Вплив силікату кальцію, у-аміномасляної кислоти (ГАМК) та їх суміші на адаптацію пшениці та кукурудзи до імітованого кислотного дощу було оцінено у вегетаційних дослідах. Кислотні опади моделювали дворазовим поливом дистильованою водою підкисленою сірчаною кислотою до рівня pH = 2. Тест-рослини вирощували у фітокамері за контрольованих умов температури, освітлення, відносної вологості протягом 18 діб. Фізіологічний стан тест-рослин оцінювали за показниками росту (висота надземних частин, довжина коренів, суха маса надземних частин та коренів), вмістом фотосинтетичних пігментів, флавоноїдів та проліну в листках. Для кукурудзи оцінювали також вміст антоціанів в надземних частинах та коренях. Паралельно визначали фізичні та хімічні характеристики ґрунту: pH, електропровідність, окисно-відновний потенціал, вміст розчинних карбонатів та нітратів.

Встановлено, що імітовані кислотні опади пригнічували ріст та вміст фотосинтетичних пігментів в листках пшениці та кукурудзи. Вміст захисних метаболітів (проліну, флавоноїдів та антоціанів) зростав. Пшениця проявила більшу чутливість до пригнічуючого впливу кислотних опадів порівняно з кукурудзою. Внесення CaSiO₃ більш ефективно впливало на відновлення рН та концентрації аніонів HCO₃⁻ у ґрунті, ніж ГАМК. Тоді як внесення ГАМК більш ефективно сприяло акумуляції аніонів NO₃⁻ у ґрунті. Суміш CaSiO₃ та ГАМК була найбільш ефективною у відновленні фізико-хімічних властивостей ґрунту, змінених імітованим підкисленням, а також у стимуляції росту та фотосинтезу досліджуваних рослин. Таким чином, суміш CaSiO₃ та ГАМК є перспективною для подальших досліджень можливості її застосування для пом'якшення негативного впливу кислотних опадів на рослинність та ґрунт.

Ключові слова: кислотний дощ, силікат кальцію, γ-аміномасляна кислота, кукурудза, пшениця, ріст, фотосинтетичні пігменти, пролін, флавоноїди, антоціани