Oxygen enrichment with magnesium peroxide for minimizing hypoxic stress of flooded corn

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Abstract

Flooding/waterlogging is a major factor responsible for hypoxic stress in agriculture. The aim of this study was to develop an effective oxygen buffer with magnesium peroxide (MgO2) to generate hydrogen peroxide (H2O2) and release bioavailable oxygen. MgO2 provided a relatively stable level (approx. 300 μM) of bioavailable oxygen. The oxygen-buffer system is adjustable and controllable by adding Mg2+ or EDTA to the aqueous system. Regular H2O2 was also able to provide bioavailable oxygen but the system was poorly buffered with respect to oxygen release. The accessibility of plants to bioavailable oxygen was indicated by the activity of alcohol dehydrogenase (ADHase, EC 1.1.1.1), an anaerobically induced enzyme of flooded plants. The application of MgO2 to flooded soil reduced ADHase activity in corn-root tips by 91.3%. This application of MgO2 presents a novel pathway to significantly (P < 5%) minimize adverse impacts of hypoxia on flooded corn seedlings. This finding may have broad implications for addressing hypoxic problems in crop science and technology.

Key words: alcohol dehydrogenase activity / hypoxic stress / magnesium peroxide / oxygen buffer solution / Zea mays L.

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1 Introduction

The USA produces 41% of the world’s corn (Zea mays L.), which is more than twice the production of any other country (FAO, 2009). Corn-planted area in the USA was estimated at 39 million hectares in 2012 (USDA, 2012). Corn has many uses including human consumption as sweet corn, starch, oil, sweeteners, grits, corn flour, cornmeal, and beverage alcohol (Curic et al., 2009; Bandeira et al., 2012). Corn is also prominent in livestock feed, bio-ethanol production, and world trade (Reddy et al., 2008). Florida has been the principal state for sweet corn production in the USA since the 1980s (Debra, 2003). As energy prices have escalated, the use of corn for bioethanol has also developed rapidly increasing the demand for corn even further (Vadas et al., 2008; Shrestha et al., 2012). However, a challenge to corn production is oxygen deficiency, namely hypoxia that can occur when heavy rains in the US Corn Belt or tropical storms and hurricanes in Florida occur soon after seeding and during early vegetative stages.

Corn is highly susceptible to waterlogging damage at early vegetative stages. This stress results in severe reductions of plant canopy height, dry matter production, and yield (Mukhtar et al., 1990; Rao and Li, 2003; Rosenzweig et al., 2002). In 2008, floodwater from spring rains caused 100% crop loss in the impacted croplands of Illinois, Missouri, and Indiana (Olson, 2009). Corn yield loss in the USA caused by excess precipitation is significant and may double during the next 30 years (Rosenzweig et al., 2002). The annual monetary losses from flooding may increase by up to US $1.5 billion by 2100 (Wobus et al., 2013). The primary cause of environmental stress from flooding is an insufficient oxygen concentration in the soil, because the oxygen diffusion coefficient in air (2.14 × 10−1 cm2 s−1) is 10.000 times greater than that in water (1.97 × 10−5 cm2 s−1; Holbrook and Zwieniecki, 2003). Thus, corn plants in wet soils receive insufficient O2 for normal root metabolism. This flooding/waterlogging stress has been challenging agricultural scientists for decades (Crawford, 1967; Xie et al., 1989; Hwang and VanToai, 1991; Dennis et al., 2000; Nguyen et al., 2012). It is imperative to employ new strategies for mitigating harmful effects of hypoxic stresses resulting from flooding and waterlogging in crop production.

Physiologically, O2 is a terminal electron acceptor in the mitochondrial electron transport chain and oxidative phosphorylation and, thus, enables plants to generate sufficient chemical energy (stored as the coenzyme ATP) needed for intracellular physiological and biochemical reactions. Flooding results in O2 deficiency and lack of electron acceptors in respiration.

Magnesium peroxide is a strong oxidizer that has a great potential to be used as a soil additive to increase soil O2 and reduce plant stress from flooding. The solubility of MgO2 in deionized (DI) water is 7.42 × 10−10 M (Waite et al., 1989). Every mole of MgO2 can slowly produce one mole of hydrogen peroxide (H2O2) in water. Every mole of H2O2 produced...
can, in turn, generate one-half mole of bioavailable oxygen. Magnesium peroxide can be easily applied and incorporated into the soil because MgO$_2$ is a solid. Magnesium peroxide may provide new avenues for alleviating root hypoxia resulting from flooding for up to 300 d (Borden et al., 1997; Golberg et al., 2003).

Alcohol dehydrogenase (ADHase; EC 1.1.1.1) is one of the enzymes for fermentation known to be biosynthesized preferentially under hypoxia (Andrew et al., 1993). Under normoxic conditions, 1 mole of glucose is broken down into 2 moles of pyruvate in aerobic glycolysis. Pyruvate is shuttled into the mitochondria and converted to carbon dioxide, producing water and 36 to 38 moles of ATP, which is most important for physiological and biochemical reactions in corn seedlings (Rich, 2003; Strommer, 2011). Under aerobic conditions, oxygen—as an electron acceptor—is sufficient for oxidative respiration and no ADHase is needed (Irfan et al., 2010). However, sufficient O$_2$ for aerobic respiration is not available when corn seedlings are flooded (Liu et al., 2012a). Under flooded or other hypoxic conditions, corn seedlings may become modified anatomically by developing aerenchyma that deliver O$_2$ from exposed leaves to flooded parts (Bailey-Serres and Voesenek, 2008; Drew et al., 2000), or shifting from aerobic to anaerobic glycolysis, i.e., fermentation. Corn plants’ aerobic patterns of gene expression such as transcription and translation stop under hypoxia (Strommer, 2011). Consequently, anaerobic patterns of gene expression start and anaerobic enzymes such as ADHase are preferentially produced (Okimoto et al., 1980; Strommer, 2011).

Gaseous O$_2$ is difficult to be delivered into flooded soil but solid oxygen fertilizers such as MgO$_2$ are not. Our hypothesis is that solid oxygen fertilizers can be easily applied to flooded soil and provide O$_2$ bioavailable for flooded corn plants. Due to its poor solubility, MgO$_2$ releases bioavailable O$_2$ as long as 300 d (Borden et al., 1997; Golberg et al., 2003). This long duration of O$_2$ availability can offer us a novel approach to alleviate hypoxic problems from flooding and waterlogging. The roots of flooded corn treated with MgO$_2$ were expected to have better root metabolism and lower ADHase activity as compared to the control without oxygen fertilization.

The objectives of this study were: (1) to develop an O$_2$ buffer system with MgO$_2$ that can be used to increase oxygen bioavailability in flooded soil, (2) to assess the adjustability of bioavailable oxygen in the buffer system by adding or removing Mg$^{2+}$ ions, and (3) to estimate the effect of MgO$_2$ application on ADHase activity in flooded corn seedlings in a container-based study.

### 2 Theory for establishing an O$_2$ buffer with poorly soluble MgO$_2$

When in contact with water, MgO$_2$ decomposes by the following chemical reactions and releases H$_2$O$_2$ into water:

\[
\text{MgO}_2(s) + 2\text{H}_2\text{O}(l) \rightarrow \text{Mg(OH)}_2(s) + \text{H}_2\text{O}_2(aq). \quad (1)
\]

Then, the newly produced H$_2$O$_2$ can be further decomposed to H$_2$O and oxygen, bioavailable for plants to use when contacted with catalase from root exudates and bacteria in the solution:

\[
2\text{H}_2\text{O}_2(l)_{\text{catalase}} \rightarrow 2\text{H}_2\text{O}(l) + \text{O}_2(aq). \quad (2)
\]

According to K$_{sp}$(MgO$_2$) = 5.5 x 10$^{-11}$, in Eq. 1 [Mg$^{2+}$] = [H$_2$O$_2$] = 7.42 x 10$^{-6}$ M (Waite et al., 1999). Based on its K$_{sp}$, and a molar mass of 56.3 g mol$^{-1}$, the solubility of MgO$_2$ is 4.18 x 10$^{-4}$ g L$^{-1}$. Because the reaction constant at Eq. 2 is greater than 100 (Melnik et al., 1979), the actual bioavailable oxygen (namely dissolved oxygen) concentration can be more than 100-fold greater than the actual concentration of H$_2$O$_2$ generated from MgO$_2$ in contact with water. Catalase (EC 1.11.1.6) in Eq. 2 was expected to be existent in the corn root exudates and possible bacteria in the solution because nearly all living organisms possess the enzyme (Chelikani et al., 2004; Xie et al., 2012).

Magnesium ions (Mg$^{2+}$) can effectively adjust H$_2$O$_2$ concentrations in an aqueous solution and, therefore, O$_2$ concentration can, in turn, be controlled. Any mechanism or pathway that removes Mg$^{2+}$ and H$_2$O$_2$ from the aqueous solution will facilitate O$_2$ release from MgO$_2$. For example, uptake of Mg$^{2+}$ by plants can accelerate O$_2$ release from MgO$_2$. Also, cations can be chelated if a chelator, such as EDTA, is added into the system. These Mg$^{2+}$ reductions are all able to boost dissolution of MgO$_2$. However, if external Mg$^{2+}$ is added to the solution, the equilibrium will be shifted, reducing the bioavailable O$_2$ release from MgO$_2$. In this case, MgO$_2$ is the reservoir of bioavailable O$_2$ for flooded plants. Magnesium peroxide can be used to create an adjustable O$_2$ buffer system for plants or other aerobic organisms.

### 3 Material and methods

#### 3.1 Material

Corn (Zea mays L. cv. FR27 × FRM017) seeds were provided by Illinois Foundation Seeds, Inc. (Geneseo, IL, USA). All chemicals were from Sigma-Aldrich (St. Louis, MO, USA) except MgO$_2$ as a mixture of 35% MgO$_2$, 60% MgO, and 5% Mg(OH)$_2$ from Solvary Interox, Inc. (Houston, TX, USA), and food grade 3% H$_2$O$_2$ from Aaron Industries, Inc. (Clinton, SC, USA).

#### 3.2 Aeroponic culture of corn seedlings

Seeds were germinated and grown aeroponically in a growth chamber (Percival model I-36VL, Percival Scientific, Inc., Perry, IA, USA) at 25 ± 1°C, 16 : 8 h light : dark photoperiod, and 60% relative humidity. The composition of the 100% strength nutrient solution was as follows: 1 mM NH$_4$NO$_3$, 0.2 mM Na$_2$PO$_4$, 1 mM K$_2$SO$_4$, 2 mM CaCl$_2$, 3 mM MgSO$_4$, 0.2 μM H$_3$BO$_3$, 0.2 μM CuSO$_4$, 0.01 μM (NH$_4$)$_6$Mo$_7$O$_24$, 5 μM MnSO$_4$, 0.2 μM ZnSO$_4$, 200 μM Fe-EDTA (Yan et al., 1998), and 0.36 μM Na$_2$SiO$_4$ as sodium silicate (Epstein, 1994). The aeroponic system was from Growgenie (Growgenie, San Jose, CA, USA); 25 L of nutrient solution were poured into a 38 L square tank (61 cm × 61 cm × 30 cm). The tank was covered with a 5 mm thick plastic sheet containing 60 evenly
spaced each with a 48 mm diameter. Sixty plastic baskets (each 50 mm high with an external diameter of 55 mm at the top and 37 mm at the bottom) were suspended in the space above the solution (Liu et al., 2012a). Two corn seeds were placed into each basket. After germination, seedlings were thinned to one per basket. A 24 W electric pump (Danner Manufacturing Inc., Islandia, NY, USA) was installed at the base on the tank bottom and a nozzle was attached to the pump and positioned in the center of the tank above the surface of the water. The nozzle created a mist for treating the seeds and seedlings in the baskets. After 8 d of aeroponic growth, corn seedlings were transferred to solid substrates or to nutrient solutions, respectively.

3.3 Flooding treatments

The germinated seeds were individually placed into PREMIER® Pro-Mix® Ultimate Organic Potting Mix (Tindara, LLC, Georgetown, MA, USA) in 3.8 L pots, grown for 10 d, and then flooded with water to a depth of 5 cm above the potting medium surface for 7 d. The shoots of the flooded plants were elevated above the water surface. The seedlings were kept in the same type of growth chamber with the same settings as used during germination. Treatments consisted of amending the potting medium with different amounts of MgO2 incorporated into the Potting Mix before planting. The treatments were: 0, 5250, 7000, 8750, or 10500 mg MgO2 per pot. A control (CK) was paralleled without flooding.

3.4 Preparation of O2 buffer systems and analysis of O2 release from MgO2 in water or nutrient solutions

One hundred seventy five (175) mg of MgO2 were added into a polypropylene tube containing 50 mL of DI water or each of the nutrient solutions with different strengths to establish various O2 buffer systems. The strength of the nutrient solutions was 25%, 50%, 100%, 200%, or 400% of Yan’s formula (Yan et al., 1998). The O2 buffer systems were allowed to equilibrate overnight at 25±1°C before measurements were made. After 1 week of growth, when the corn seedlings reached approx. a three-leaf stage, a single seedling was transferred into the 50 mL tubes containing different nutrient strengths and different oxygen concentrations. The O2 concentration in the solution was measured with a root oxygen bioavailability sensor (see the details in the oxygen analysis section) and recorded every 5 min. The leaves of the seedlings were illuminated by a fiber light source (Model 180, 200 W, Dolan-Jenner Industries, Inc. Boxborough, MA, USA) at a PAR of 87.5 μmol photon m–2 s–1.

3.5 Adjustments of O2 release

Bioavailable oxygen concentrations in the 50 mL culture solutions were separately varied using either EDTA as Na2EDTA at one of five final concentrations (0, 2, 4, 8, and 16 mM) or Mg2+ as MgSO4 at one of six final concentrations (0, 6, 12, 18, 24, and 30 mM) in the solutions with MgO2. The solutions were equilibrated overnight at 25±1°C before the bioavailable O2 concentrations were analyzed.

3.6 Oxygen analysis

Bioavailable O2 concentrations were determined using a root oxygen bioavailability sensor constructed with a gold wire as the cathode and a silver wire as a reference (Liao et al., 2004) and a self-referencing electrochemical microelectrode (SREM) system (Applicable Electronics, Inc., Forestdale, MA, USA). Calibration was done by aerated DI water with N2 gas or with air to obtain O2 concentrations of 0% (anoxic) or 21% (normoxic) concentration at 25±1°C. The values were then converted to bioavailable micromolar O2 concentrations.

3.7 ADHase activity

After seedlings had been flooded for 7 d, 2 cm of corn root tips were individually collected from plants in each treatment, cleaned with DI water, and frozen in a refrigerator at −20°C for analyses of ADHase activity. The enzyme was assayed as described by Chung and Fertl (1999) and modified slightly as follows. ADHase was extracted in an extraction buffer including 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 12 μM mercaptoethanol, and 0.05 mg DTT mL–1. The frozen root tips were ground rapidly in a chilled mortar and pestle with the above mentioned extraction buffer chilled at 4°C. The homogenate was centrifuged at 15000 g at 4°C for 15 min. One hundred μL supernatant were added to the 800 μL reaction solution containing 50 mM Tris-HCl buffer (pH 9.0), 1 mM EDTA, and 1 mM NAD+ (oxidized form of nicotinamide adenine dinucleotide). The assay used 100 μL of 15% (v/v) ethanol as the substrate and measured the production of NADH (reduced form of nicotinamide adenine dinucleotide). NADH formation was measured at 340 nm in a spectrophotometer (DU 64, Beckman Instruments, Fullerton, CA, USA) for 60 s with three replications. A unit of ADHase is defined as the production of 1 nmol of NADH (mg protein · min)–1. The activity was calculated using a value of 6.22 mM–1 cm–1 as the molar extinction coefficient of NADH at 340 nm (Liu et al., 2012a; Schomburg et al., 2012).

3.8 Protein assay

Protein concentration was determined colorimetrically according to Lowry’s method to read the absorbance at 750 nm with three replications (Lowry et al., 1951; Peterson, 1977). Ten μL of the aforementioned supernatant for ADHase activity analysis were mixed with 990 μL of Lowry A reagent containing equal volumes of the following three solutions: (1) copper-tartrate-solution (0.1% CuSO4, 0.2% KNa-tartrate, and 10% NaCO3 in DI water), (2) 10% sodium dodecyl sulfate, and (3) 0.8 N NaOH. After 15 min, 500 μL of Lowry B solution (one part of 2 N Folin & Ciocalteu’s Phenol Reagent Solution (Sigma) diluted in five parts of DI water) were added. Bovine Serum Albumin was used to prepare the standards (Liu et al., 2012a).
3.9 Statistical analyses

All of the analyses had three replications. The data were statistically analyzed by using SAS 9.12 software (SAS Institute, Cary, NC). The analysis of variance (ANOVA) was based on a randomized complete block design. Means were separated using the Least Significant Difference (LSD). The critical ranges (LSD_{0.05, 2}) of Duncan’s Multiple Range Test at P < 5% were used to identify significant differences among the means (Hubbard, 2001).

4 Results

4.1 Oxygen bioavailability of culture solution with MgO2

When 175 mg MgO2 were added to 50 mL of culture solution, the O2 bioavailability was always significantly greater than that of the control without MgO2 no matter what nutrient strength of the solution was used. However, the bioavailable concentration of oxygen released from MgO2 in culture solution was significantly decreased with increase of nutrient strength. There was no significant difference in oxygen bioavailability in the culture solution without MgO2 regardless of nutrient strength (Fig. 1).

4.2 Oxygen bioavailability adjustment of aqueous systems with MgO2 and Mg2+ ions

After EDTA had been added into the aqueous solution at final concentrations of 2, 4, 8, or 16 mM, the overall O2 bioavailability in the solution was increased by 11, 27, 47, or 83%, respectively. The concentration of bioavailable O2 was significantly increased when the added EDTA concentration was equal to or greater than 4 mM (Fig. 2A). The bioavailable O2 concentration was significantly reduced by 2, 18, 21, 25, and 22% through increasing Mg2+ ion concentrations to 6, 12, 18, 24, and 30 mM, respectively (Fig. 2B).

4.3 Oxygen buffering capacity of aqueous systems with or without MgO2

As respiration of the corn root progressed, bioavailable O2 in the culture solution was consumed. When equilibrated overnight at 25 ± 1°C, a 200%-strength complete nutrient solution (CNS) had approx. 260 μM of bioavailable oxygen (Fig. 1). In 50 mL of the culture solution there was a total of approx. 370 μg bioavailable oxygen without MgO2. This small amount of oxygen was consumed in approx. 60 min (curve for CNS in Fig. 3).

Hydrogen peroxide (H2O2) contains 47% bioavailable oxygen on a weight basis. When 1 mL of 3% H2O2 was added to the 50 mL culture solution, it contained 0.06% H2O2; the bioavailable O2 concentration was strongly increased and reached roughly 700 μM during the first hour of plant growth (curve for H2O2 in Fig. 3). This level of bioavailable oxygen was two-fold greater than that of the culture solution that had been equilibrated overnight. The increase of O2 probably resulted from the catalyzed decomposition of H2O2 by the catalase from the corn root exudates and bacteria. After reaching a maximum at approx. 35 min, the O2 concentration in the solution decreased to 230 μM within 2 h of plant growth. After 3 h in the presence of actively growing corn roots, the bioavailable O2 in solution was mostly consumed. However, when 175 mg MgO2 were added to the culture solution (curve for MgO2 in Fig. 3), the bioavailable O2 concentration increased within the first hour and remained stable thereafter. It was still as high as 320 μM after 3 h following addition of corn seedlings,
which is approx. 90 μM greater than that of the overnight equilibrated CNS.

4.4 Oxygen depletion of culture solutions with MgO₂ and EDTA

When 50 mL of 200% strength culture solution were supplemented with 175 mg MgO₂ and 10 mM EDTA, the bioavailable O₂ concentration (Fig. 4) was greater than that of the same culture solution without EDTA (curve for MgO₂ in Fig. 3). The peak of the O₂ bioavailability curve in Fig. 4 lasted much longer than that of the curve for H₂O₂ in Fig. 3. These differences were attributed to the chelation of Mg²⁺ by EDTA, which caused more MgO₂ to be dissolved and to release additional bioavailable O₂. When the seedling was taken out of the solution for 5 min, the concentration of bioavailable O₂ was increased by 24.5% from 229 to 285 μM. The average increase of O₂ concentration was 11.2 μM min⁻¹ during that 5 min time window without an actively growing plant in the system (Fig. 4).

4.5 Effects of MgO₂ on ADHase activity in flooded corn root tips

After growing for 10 d in an aerobic soil condition, corn seedlings were flooded, and the O₂ bioavailability effects on ADHase activity were found to vary with MgO₂ levels. Activity of ADHase decreased significantly with increasing application rates of MgO₂ (Fig. 5).

5 Discussion

This study demonstrates that MgO₂ can effectively provide bioavailable O₂ to plant roots under flooded conditions, and...
5.1 Oxygen bioavailability of culture solution with MgO$_2$

The liberation of bioavailable O$_2$ from MgO$_2$ was decreased by adding Mg$^{2+}$ ions into the culture solution (Fig. 2B). Other nutrients also contributed to the decrease in bioavailable O$_2$ release from the MgO$_2$ in the solution (Fig. 1), because—based on chemical thermodynamics—the internal energy in a culture solution is lower than that in DI water (Stumm and Morgan, 1996). This significant difference in O$_2$ bioavailability between the two types of culture solutions indicated that MgO$_2$ significantly enriched the O$_2$ level in the culture solution.

5.2 Oxygen bioavailability enrichment of aqueous systems with MgO$_2$

The results from this study showed that MgO$_2$ was effective for creating different levels of bioavailable O$_2$ in various controllable oxygen-release systems. Oxygen buffer systems in aqueous solutions can be established by using MgO$_2$ with culture solution (Fig. 1). As Mg is an element essential for plant growth and development, corn seedlings themselves can positively adjust O$_2$ bioavailability by taking up Mg$^{2+}$ and, thus, affecting MgO$_2$ solubility in the aqueous system, although this aspect was not investigated in this study. Furthermore, plant root exudates have chelating properties (Marschner et al., 1989), and the chelation of Mg$^{2+}$ would facilitate MgO$_2$ solubility and hence increase O$_2$ bioavailability.

5.3 Oxygen buffering capacity of aqueous systems with or without MgO$_2$

Hydrogen peroxide can release 47% bioavailable oxygen after decomposition. The culture solution contained 0.06% H$_2$O$_2$ after adding 1 mL of 3% H$_2$O$_2$ to a 50 mL culture solution. The bioavailable O$_2$ concentration was significantly increased (curve for H$_2$O$_2$ in Fig. 3). This concentration of bioavailable O$_2$ dropped down quickly and the O$_2$ in solution was mostly consumed within 2.5 h. These rapid fluctuations indicate that H$_2$O$_2$ is poor in O$_2$ buffering capacity. However, when 175 mg MgO$_2$ were added to the culture solution (curve for MgO$_2$ in Fig. 3) the bioavailable O$_2$ concentration then remained fairly steady at approx. 320 μM. After 3 h of O$_2$ depletion by the three-leaf corn seedling, the bioavailable O$_2$ was still greater than 320 μM, which is greater than that in air-saturated DI water. These data are interpreted to mean that a culture solution with MgO$_2$ can serve as an O$_2$ buffer system. This O$_2$ buffer may provide us a novel approach to deal with flooding/waterlogging or other hypoxic stresses that plants have to face.

5.4 Oxygen depletion of culture solutions with MgO$_2$ and EDTA

Culture solutions with MgO$_2$ have much stronger buffering capacity than those with H$_2$O$_2$. The O$_2$ depletion curves in Fig. 3 show the difference in O$_2$ supply to corn seedlings. EDTA can facilitate the dissolution of MgO$_2$ as it chelates metals, i.e., magnesium in this case (Holleman and Wiberg, 2001; Prywer and Olszynski, 2013). MgO$_2$ can release bioavailable O$_2$ faster when EDTA is added to the system. Thus, the combination of EDTA and MgO$_2$ can make the O$_2$ buffer even stronger (Fig. 2). Root exudates contain chelates as well (Marschner, 1989) and may also accelerate O$_2$ release from MgO$_2$ when applied to culture solution or soil. No related research has been done yet. Based on the two equations in the theory section of this article, MgO$_2$ produces two moles of magnesium hydroxide [Mg(OH)$_2$] per mole of O$_2$ release. Mg(OH)$_2$ has a K$_{sp}$ of 5.61 × 10$^{-12}$ (Lide, 1998) and can release 2.4 μM Mg$^{2+}$ ions. K$_{sp}$[Mg(OH)$_2$] is approx. 10-fold smaller than K$_{sp}$[Mg(O$_2$)]. The formation of Mg(OH)$_2$ can facilitate O$_2$ release from MgO$_2$. Thus, the aforementioned chelates may benefit O$_2$ fertilization to flooded plants. Bacteria and fungi may also promote O$_2$ release from MgO$_2$ because they can produce chelates as well (Kosman, 2013).

5.5 Effects of MgO$_2$ on ADHase activity in flooded corn root tips

ADHase plays a key role in anaerobic metabolism in plants as it catalyzes the reduction of acetaldehyde to ethanol. Ethanol is less toxic and more diffusible than lactic acid (Okimoto et al., 1980; Strommer, 2011). ADHase activity is usually considered essential for the survival of corn seedlings affected by flooding (Johnson et al., 1994; Chung and Ferl, 1999) and is closely related to flooding/waterlogging tolerance of plant roots (Crawford, 1967). ADHase activity significantly decreased with the application of MgO$_2$ to the potting medium because MgO$_2$ provided bioavailable O$_2$ to the flooded plants. The enzyme activity reductions indicate that MgO$_2$ may alleviate the hypoxic effects on the flooded seedlings, and likely improve their physiological and biochemical status. MgO$_2$ has a great potential to ameliorate anoxic or hypoxic challenges to horticulture and probably to agriculture and forestry as well. The use of MgO$_2$, as shown here, can supply bioavailable O$_2$ continuously for as long as 300 d (Borden et al., 1997; Goldberg et al., 2003). Currently, however, pure MgO$_2$ is not readily available from commercial sources. For example, Sigma Company supplies only 28% MgO$_2$. The impurities in commercially available peroxides may cause difficulties for control of O$_2$ release and may have hindered earlier work. Purer sources of poorly soluble peroxides would greatly facilitate their potential commercialization in the future.

5.6 Application prospect of MgO$_2$ to flooded corn

Traditionally, the strategies dealing with flooding/waterlogging are to improve the ability of crops to tolerate hypoxic stresses.
Wild species are usually used as genetic sources of flooding tolerance traits for plant breeding programs (Harlan, 1976; Ali et al., 2010). The wild relatives of corn are referred to as “teosinte” (Abiko et al., 2012). This group is reputed to have greater tolerance to waterlogging than other species of Zea (Iltis and Benz, 2000). These wild species have been used for corn breeding programs (Mano and Omori, 2009). This biological strategy usually needs years to complete the genetic modification of corn cultivars to have the tolerance traits. The logical strategy usually needs years to complete the genetic modification of corn cultivars to have the tolerance traits. The application of MgO$_2$ is a chemical strategy and can substantially improve soil redox potential (Liu et al., 2012b). After release of bioavailable O$_2$, MgO$_2$ is turned into Mg(OH)$_2$, which can release Mg$^{2+}$ and OH$^-$ ions (Eq. 1). The application of MgO$_2$ to hydroponics and soil both worked well in this research. More research is, however, needed for the MgO$_2$ application to field scale and for other crops because there is no information available for the influence on agro-ecosystems.

6 Conclusions

This study created a new concept of O$_2$ fertilization and provided a novel pathway to improve the redox potential of flooded soils and to alleviate hypoxic stress caused by flooding/waterlogging. As a slightly soluble chemical, MgO$_2$ can keep releasing bioavailable oxygen for as long as 300 d. The bioavailability of O$_2$ can be increased or decreased by adding EDTA or Mg$^{2+}$ ions, respectively. The O$_2$ bioavailability in culture solutions with MgO$_2$ is controllable and adjustable by increasing or decreasing Mg$^{2+}$ concentrations. The “solid oxygen fertilizer” is deliverable in water or flooded soil. However, field tests need to be conducted not only on the efficacy of the MgO$_2$ for increasing O$_2$ in flooded soils in field scales, but also to determine if there are unanticipated effects in the agro-ecosystem.

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