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Characteristics and mechanisms of acrylate polymer damage to maize seedlings

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ABSTRACT

Superabsorbent acrylate polymers (SAPs) have been widely used to maintain soil moisture in agricultural management, but they may cause damage to plants, and the mechanisms are not well understood. In this study, seed germination, soil pot culture, hydroponic experiments, and SAPs degradation were conducted to investigate damage characteristics and mechanisms associated with SAPs application. The Results showed that SAPs inhibited maize growth and altered root morphology (irregular and loose arrangement of cells and breakage of cortex parenchyma), and the inhibitory effects were enhanced at higher SAPs rates. After 1 h SAP hydrogels treatment, root malondialdehyde (MDA) content was significantly increased, while superoxide dismutase (SOD) and catalase (CAT) content were significantly decreased. Hydroponics experiment indicated that root and shoot growth was inhibited at 2.5 mg L^{-1} acrylic acid (AA), and the inhibition was enhanced with increasing AA rates. This effect was exacerbated by the presence of Na^+ at a high concentration in the hydrogels. Release and degradation of AA were enhanced at higher soil moisture levels. A complete degradation of AA occurred between 15 and 20 days after incubation (DAI), but it took longer for Na^+ concentration to decrease to a safe level. These results indicate that high concentration of both AA and Na^+ present in the SAPs inhibits plant growth. The finding of this study may provide a guideline for appropriate application of SAPs in agriculture.

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1. Introduction

Water availability is of vital importance to plant growth. Both drought and flooding, which occur more frequently due to global warming, threaten agricultural production and food security. Numerous agricultural practices have been adopted to increase soil water holding capacity (WHC) so that crop production can be sustained under water stress condition and soil erosion and runoff can be reduced. Recently, super absorbent polymers have been increasingly used in agricultural and horticultural practices to improve soil WHC. Superabsorbent acrylate polymers (SAPs) can absorb water up to several hundred times of its own weight (Han et al., 2010; Yu et al., 2012; Zhong et al., 2013), which greatly increases soil WHC and reduces nutrient loss (Mao et al., 2006; Eneji et al., 2013; Li et al., 2014). Ninety percent of the retained water in the SAPs is plant available (Huang et al., 2002), thus greatly promoting plant growth under drought and semi-drought conditions (Arbona et al., 2005; Abedi-Koupai and Asadkazemi, 2006; Apostol et al., 2009; Orikiriza et al., 2013; Liu and Chan, 2015). Therefore, SAPs are also increasingly used in agriculture to enhance use efficiency of fertilizers.

However, detrimental effects of SAPs on seed germination and plant growth have been increasingly reported (Ingram and Yeager, 1987; Austin and Bondari, 1992; Cui and Li, 2000). Water deficiency aggravated the inhibition of SAPs to plant growth, especially at a high dose (Tripepi et al., 1991; Islam et al., 2011). It was proposed that SAPs might compete with plant roots for water, thus reducing water availability to plant growth (Zhang and Ma, 2008; Lv et al., 2009). However, this allegation contradicts with the fact that 90% of water retained in SAPs is available to plants before the wilting point is attained (Huang et al., 2002, 2003; Huang and Xia, 2005). Our previous studies found that the inhibitory effects of SAPs are related to some chemical substances present or released during SAP decomposition that are phytotoxic.

Most of the SAPs used in agriculture are polymers derived from acrylic acids (AA) (Zhang et al., 2005). During the synthesis of SAPs, sodium hydroxide (NaOH) or potassium hydroxide (KOH) is used to neutralize AA (Zohuriaan-Mehr and Kabiri, 2008). The final products of SAPs may contain 16% Na^+ when 70% AA is neutralized with NaOH, and the concentration of unconsumed free AA may be as high as 1500 mg kg^{-1} (Fiume, 2002). Although AA has low toxicity to animals (Staples et al., 2000), its toxicity to plants

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has not been reported yet.

The objectives of this study were to characterize the nature and elucidate the mechanism of plant damage associated with SAPs applications.

2. Materials and methods

2.1. Superabsorbent acrylate polymers (SAPs)

Characteristics of SAPs (Guangzhou, China): retention capacities in deionized (DI) water and 0.9%NaCl solution were 391.1 and 43.82 g g^{-1}, respectively, and the contents of free AA and Na^+ were 0.5625% and 16.54%, respectively.

2.2. Effects of SAPs on maize growth

Soil characteristics: the physio-chemical properties of soil (vegetable cultivated red soil) include: pH 6.85, organic matter 16.79 g kg^{-1}, total N 0.46 g kg^{-1}, total P 0.54 g kg^{-1}, total K 9.83 g kg^{-1}, available N 75.6 mg kg^{-1}, available P 12.6 mg kg^{-1}, exchangeable K 106.0 mg kg^{-1}, exchangeable Na 28.1 mg kg^{-1}, exchangeable Mg 97.4 mg kg^{-1}, exchangeable Ca 2023 mg kg^{-1}.

Experimental setup: In pot experiment, SAPs were applied at four levels: 0%, 0.1%, 0.3% and 0.6% of dry soil (denoted as CK1, L, M, and H), with 6 replicates for each treatment. For each pot, 5 kg soil was mixed with 1.31 g urea, 1.22 g ammonium phosphate monobasic ($NH_4H_2PO_4$), and 1.25 g potassium chloride (KCl). The soil water was 65% of WHC and soil water potential (ψ_{ck}) was monitored throughout the experiment with tensiometers (TDR100/200).

Three germinated maize (*Zea mays*) seeds were sown to each pot. Maize seedlings were harvested at the 15th and 40th day after planting (DAP), respectively, and shoot and root biomass were recorded. At the 15th DAP, root tip in close contact with hydrogels was collected and analyzed for root morphology using a scanning electron microscopy (SEM) (Model S-3700N, Hitachi, Japan). To prepare root samples for SEM analysis, fresh roots were rinsed with DI water, and 1–2 cm root tip was cut and immersed into agar briefly. The root tip was then cut with a double-edge razer and freeze-dried in liquid nitrogen for 24 h. The samples were then carbon coated and scanned with the SEM.

2.3. Effects of SAPs on seed germination and seedling physiology

Seed germination: maize seeds were disinfected in 0.1% HgCl solution for 10 min and rinsed with DI water. The treated seeds were then blotted dry with paper towel. Thirty seeds were placed onto 500 g SAPs hydrogels absorbing 50, 100, 200 and 300 g DI water g^{-1}, denoted as A1, A2, A3 and A4, respectively. Wetted sand was used as control (CK2). The planted seeds were covered with a wet filter paper. The containers with seeds were incubated in darkness in a 25 ± 1 °C incubator. The germination energy was determined at the 4th DAP; the germination rate, plant height and fresh biomass were determined at the 7th DAP.

Seedling physiology: the hydrogel treatments include DI water as control (CK3) and four levels of water content, namely 50, 100, 200 and 300 g g^{-1}, denoted as B1, B2, B3 and B4, respectively. 70 seedlings at two-leaf stage were planted in the hydrogels and incubated in the illuminated incubators for 1 h. 35 treated seedlings were harvested and rinsed with DI water and the 1–4 cm root tips were collected for determination of superoxide dismutase (SOD) and catalase (CAT). After 48 h incubation, the remaining 35 plants were harvested for determination of root vigor and malondialdehyde (MDA). Measurements of root vigor, MDA, SOD and CAT were according to the TTC method, thiobarbituric acid reactive substances assay (TBARS), nitro blue tetrazolium assay, and ultraviolet light absorption method, respectively.

2.4. Effects of AA and Na on maize seedlings

The hydroponics experiment was a two way factorial design, with two levels of Na (0 and 4000 mg L^{-1}) and four levels of AA (0, 2.5, 5 and 10 mg L^{-1}). The experiment consisted of eight treatments: CK4 (control without NaCl or AA), N (NaCl 4000 mg L^{-1}), LA (AA 2.5 mg L^{-1}), MA (AA, 5 mg L^{-1}), HA (AA, 10 mg L^{-1}), LAN (AA 2.5 mg L^{-1} + NaCl 4000 mg L^{-1}), MAN (AA 5 mg/L + NaCl 4000 mg L^{-1}), HAN (AA 10 mg L^{-1} + NaCl 4000 mg L^{-1}). Each treatment was replicated three times. 1 cm long maize seedlings were immersed in the nutrient solution. In the first five days, the container was filled with 10 L ¼ strength Hoagland solution. The composition of the nutrient solution was: KNO_3 506 mg L^{-1}; $Ca(NO_3)_2 \cdot 4H_2O$ 1180 mg L^{-1}; KH_2PO_4 136 mg L^{-1}; $MgSO_4 \cdot 7H_2O$ 693 mg L^{-1}; H_3BO_3 2.86 mg L^{-1}; $ZnSO_4 \cdot 7H_2O$ 0.22 mg L^{-1}; $CuSO_4 \cdot 5H_2O$ 0.08 mg L^{-1}; $(NH_4)_2MoO_4 \cdot 4H_2O$ 0.02 mg L^{-1}; EDTA-2NaFe (Fe 14.0%) 30 mg L^{-1}; $MnSO_4 \cdot 4H_2O$ 2.13 mg L^{-1}. The solution pH was 6.45. The nutrient solution was aerated with air for 10 min every 1 h, and kept at 25 °C. After 5 d incubation, the AA and Na^+ was introduced into the solution as per treatments, and incubated for another 10 days. At the end of the experiment, the seedlings were collected for measurement of root, stalk and root biomass. The root morphology (root length, root surface area, root volume, and root diameter and root tip quantity) was analyzed using an automatic root scanner (Model LA600 with WinRHIZO package, Regent Instruments Inc, Canada).

2.5. Degradation of AA and release of Na^+ from SAPs in soil

Four grams of SAPs (passed through 20 mesh) was sealed in a 3 cm by 3 cm nylon mesh bag (200 mesh). Each nylon bag was placed in a pot (8 cm tall; upper and lower diameter 7.5 cm and 5.5 cm) filled with 200 g soil and then covered with 600 g soil. The soil was watered to 41.3%, 25.0% and 17.5% of WHC, and the treatments were denoted accordingly as HW, MW and LW. The pots with SAPs were incubated at 25 °C, and 6 pots from each treatment were sampled at 1, 3, 5, 7, 10, 15, and 20th day after incubation (DAI). Each treatment was replicated three times. The exchangeable Na^+ content in SAPs was extracted with ammonium acetate (NH_4OAc), and analyzed using atomic absorption spectroscopy (Z-2300, HITACHI, Japan). The AA in the SAPs and soil underneath the nylon bags was extracted with 0.9% NaCl solution and measured with a high-performance liquid chromatography (HPLC; Agilent 1100, Agilent, USA) (Zhang et al., 2001): wavelength = 210 nm, with a YWG C18 column (10 μm) 300 × 3, flow rate = 1.0 ml ml^{-1}, volume ratio of 0.01 M buffer solution/DI water/CH_3OH = 10:20:70.

2.6. Statistical analysis

The data were analyzed using SAS® 9.4 program (SAS Institute, 2014) for means and standard deviations. The treatment effects in each experiment were determined with the analysis of variance (ANOVA) and treatment means were compared using Duncan's multiple range test at the significance level of $P < 0.05$.

3. Results

3.1. Maize biomass and root tip morphology

All seeds germinated at the 3rd DAP, and the growth was inhibited in all SAPs treatments at the 5th DAP. The inhibition was

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