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Article

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Lignosulfonate improves photostability and bioactivity of abscisic acid under UV radiation

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Abstract: Abscisic acid (ABA), as a commonly used plant growth regulator, is easy to be degraded and lose its bioactivity under sunshine. To select an eco-friendly and efficient photoprotectant for the improvement of photostability and bioactivity of ABA when exposed to UV light, we tested the effects of three biodegradable natural derived high polymers — sodium lignosulfonates 3A (molecular weight (MW) > 50000, degree of sulfonation (DS) 0.48), NA (20000 < MW < 50000, DS 0.7) and calcium lignosulfonate CASA (MW < 20000, DS 0.7) on the photodegradation of ABA. Both 3A, NA and CASA showed significant photo-stabilizing capability on ABA. 3A showed preferable photostabilizing effects on ABA than CASA, while NA showed an intermediate effect. That indicated lignosulfonate with high molecular weight and low degree of sulfonation had a stronger UV absorption and the hollow aggregates micelles formatted by lignosulfonate protect ABA from UV damage. Approximately 50 % more ABA was kept when 280 mg/l ABA aqueous solution was irradiated by UV light for 2 hours in the presence of 2000 mg/l lignosulfonate 3A. The bioactivity on wheat (JIMAI 22) seed germination was greatly kept by 3A, comparing to that of ABA alone. The 300 times diluent of 280 mg/l ABA plus 2000 mg/l 3A after 2 hours irradiation showed 20.8 %, 19.3 % and 9.3% more inhibition on shoot growth, root growth and root numbers of wheat seed, separately, comparing to ABA diluent alone. We conclude that lignosulfonate 3A was an eco-friendly and efficient agent to keep ABA activity under UV radiation. This research could be used in UV-sensitive and water soluble agrichemicals and to optimize the application times and dosages of ABA products.

Keywords: abscisic acid (ABA); UV light; photodegradation; lignosulfonate; shoot and root growth

1 Introduction

Most of bio-agrichemicals are susceptible to UV damage in the field and their half-lives were very short, for example, 6 hours for avermectin (*1*) and 1 hour for spinosad (*2*). Therefore, bio-agrichemicals requires more applications and high amount to ensure their effectiveness (*3*). Abscisic acid (ABA) is an effective bio-plant-growth-regulator, which could prolong florescence (*4-7*), promote differentiation of flower bud (*8-10*), enhance the growth of root and shoot (*11-13*), raise fruit coloring rate and quality (*14-19*), and improve resistance to drought stress (*20-25*). ABA also had curative effects on inflammation, type Ⅱ diabetes and tumor of animals and humans (*26-28*). ABA is a potential product that would be widely applied both in agriculture and medical care. However, due to UV light of sunshine, ABA is readily losing its bioactivity by isomerizing to inactive *trans*-ABA (Figure 1) (*29-31*). The half-life of ABA is only 24 min (*32*). This increases economic cost for ABA field application.

Many efforts have been made to keep the bioactivity of ABA and reduce its photosensitivity (*33-36*), such as replaced the double bond or hydrogen in Δ^2 (the location of double bond) of ABA side chain to benzene ring and cyclopropyl or fluorine to relieve *cis–trans* isomerization. However, the bioactivities of these new compounds were much less than that of ABA. Our previous study found benzophenone-type UV absorbers (BPs) UV-531 (2-Hydroxy-4-n-octoxy-benzophenone) and BP-4 (2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid) greatly improved the stability and bioactivity of ABA under UV radiation (*37*). However, potential endocrine disrupting effects of these UV absorbers were reported (*38-43*). They have been revealed to act in a similar way to estrogen and/or antiandrogen (*38, 39*), exert mutagenic effects on Salmonella (*40*), generate oxidative stress in Carassius auratus (*41*), suppress immunity of the immune system (*42*) and

induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovarian cells (*43*). What's worse, BPs contained in personal care products, industrial and agricultural products might pollute the aquatic environment and be bioaccumulated through food chains, as residues have been detected in water, sediments, human breast milk, urine and blood (*38*). Though the toxic of BP-4 is rather low (*39*), the potential impacts cannot be excluded. Potential environmental impacts of BPs have led to an increase concern over their usage in commercial products and their presence in the environment. Therefore, it is better to develop eco-friendly photoprotectants to alleviate the photosensitivity of ABA in application.

Lignosulfonate is sulfonated, fragmented lignin from the waste liquor of sulfite pulp mills (*44-48*) (Figure 2). It is highly cross-linked polymer formed from different phenyl-propanoid units and keeps approximately the original chemical structure of native lignin except for the introduction of a great number of sulfonic acid groups on the α-carbons of lignin side chains (*49*). The sulfonic acid groups make it easy to be soluble in the water. Due to hydrophilic groups (sulphonic, phenylic hydroxyl, carboxylic and alcoholic hydroxyl) and hydrophobic groups (aromatic, aliphatic and carbon chains), lignosulfonate showed surface activities (*44, 46, 47, 50*). Due to the existence of phenolic hydroxyl group, carbonyl and other chromophores, some researchers used lignin and lignosulfonate as sun blockers added into the emulsions of cosmetics (*51*), or as wall material of microcapsule for protecting the agrichemicals and microbial pesticide from UV damage and controlling actives release (*52-54*). The physico-chemical properties of three commercialized lignosulfonates 3A, CASA and NA were shown in Table 1. 3A has low degree of sulfonation and high molecular weight which might make it has more α-O-4 ether bonds and conjugated systems separately. CASA has low sulfonation degree and molecular weight which

might make it have fewer α-O-4 ether bonds and conjugated systems. NA has high sulfonation degree and intermediate value of molecular weight which might make it have fewer α-O-4 ether bonds and intermediate levels of conjugated systems.

We hypothesized that applying lignosulfonate could reduce photosensitivity of ABA. The objective of this study was therefore to test the hypothesis and to further select an efficient photoprotectant. The dynamics of ABA degradation at different doses of selected lignosulfonate and afterwards bioactivity of ABA plus lignosulfonate on plant growth were also analyzed. The bioactivity of lignosulfonate itself at different doses on plant growth was analyzed as well.

2 Materials and methods

2.1 Experimental design and managements

Experiment 1 was designed for comparing the stabilizing effects of three lignosulfonates on ABA degradation under UV light. Three lignosulfonates were calcium lignosulfonate Borresperse CA-SA, abbreviated as CASA; sodium lignosulfonate Borresperse NA, abbreviated as NA; and modified and purified sodium lignosulfonate 3A, abbreviated as 3A. Solid contents all above were greater than 93 % and main properties were listed in Table 1. All lignosulfonates were purchased from Borregaard LignoTech. These lignosulfonates at the dose of 0 mg/l and 500 mg/l were added into 5 mg/l ABA (pure content above 98 %, purchased from J&K Scientific Ltd.) aqueous solution. Quartz test tubes (16 mm×13 mm×150 mm, obtained from Nanjing Xujiang Electromechanical Plant) with 10 ml corresponding ABA solution prepared above were exposed to UV light in photochemical reactor (XPA-7, obtained from Nanjing Xujiang Electromechanical Plant). Test tube of each treatment covered with aluminum foil was set as dark control. Mercury lamp (300 W)

equipped with 365 nm light filter (both obtained from Nanjing Xujiang Electromechanical Plant) was applied as UV radiation source (S1), around which the test tubes could orbit. The actual irradiance is the energy of mercury lamp multiplying the transmittance of UV filter. The actual irradiance of UVA was detected as 9974 $\mu W/cm^2$, UVB as 2730 $\mu W/cm^2$. And the actual irradiance ratio of UVA to UVB was 3.7. Solutions in the tubes were stirred in order to achieve uniform irradiation. At intervals of 1 min, 0.5 ml solution of each treatment under irradiation was sampled and directly injected into high performance liquid chromatography (HPLC) after high speed centrifugation. Dark control of each treatment was determined after irradiation ended. Total radiation time lasted for 8 min and the experiments were repeated three times.

Experiment 2 was for further optimizing the dosage of lignosulfonates added into ABA application. The lignosulfonate 3A was added into ABA aqueous solution (280 mg/l). The dose of 3A was 0 mg/l, 500 mg/l, 1000 mg/l, 2000 mg/l, 4000 mg/l and 5000 mg/l respectively. Referring to the method of experiment 1, the solutions were exposed to UV light for 8 h. At intervals of 1 hour, 20 μl solution of each treatment under irradiation was sampled and injected into HPLC after diluted by 100 times and filtered by strainer (0.22 μm). Dark control of each treatment was determined after irradiation ended. The experiments were repeated three times.

Experiment 3 was for testing the remaining bioactivity of ABA in the presence of selected lignosulfonate after irradiation. In order to compare the bioactivity of ABA with and without 3A after irradiation, ABA were diluted to lower concentrations, as pre-bioassay of ABA above 2 mg/l showed badly suppression on wheat (JIMAI 22) seed germination and growth, which resulted in a difficulty to compare the differences among treatments. Therefore, ABA (280 mg/l) with and without 3A (2000 mg/l) were exposed to UV light for two hours and then diluted 300 times to

prepare 25 ml solution each and added with 20 μl Tween-20 for bioactivity assay. Petri dish (Φ = 90 mm) placed with two layers of filter paper was immersed by 4 ml each prepared solution and then seeded by twenty wheat seeds disinfected with 10 % hydrogen peroxide solution for 10 min. Five dishes were applied for each treatment as repeats. Artificial climate chest (PRX-450C, obtained from Ningbo Saifu Experimental Instrument Co., Ltd.) was used to incubate wheat seeds of all treatments in darkness at the temperature of 25 °C and humidity of 85 %. After three days' incubation, 10 seeds were chosen randomly in each dish to measure the shoot length, root length and root numbers of each treatment.

Experiment 4 was for examining the bioactivity of lignosulfonate 3A at different dosages on wheat seed germination. 25 ml lignosulfonate solution at the dose of 0 mg/l, 1.3 mg/l, 6.7 mg/l, 33.3 mg/l, 500 mg/l, 1000 mg/l, 2000 mg/l and 5000 mg/l were prepared separately for bioactivity assay referring the methods of experiment 3. After three days' incubation, 10 seeds were chosen randomly in each dish to measure the shoot length, root length and root numbers of each treatment.

Experiment 5 was for determination of critical aggregation concentration (CAC). CAC was determined with Platinum ring (ϕ = 20.5 mm × 0.6 mm) on automatic interfacial tension meter (JK99BM, obtained from Shanghai Zhongchen Digital Technic Apparatus Co., Ltd) at 20 °C. Surface tensions of lignosulfonate 3A at a series of concentration were determined and the turning point on the curve of γ-c plot was CAC. Surface tension of water was 73.7 mN/m.

Experiment 6 was for the measurements of particle size distribution of lignosulfonate 3A at a series of concentration by dynamic light scattering (DLS). The measurements were carried out by ZETASIZER Nano series (Nano - ZS90, obtained from Malvern Instruments) with a scattering

angle of 90° at 25 ± 0.01 °C. The light source is a standard laser with a power of 4 mW and a wavelength of 633 nm. The range of the particle size measurement was from 0.3 nm to 5000 nm. Accurate concentrations of lignosulfonate 3A were prepared in ultrapure water and then equilibrated for 24 h before data collection. 4 ml of each prepared solution was added into polystyrene disposable cuvette (12 mm × 12 mm × 45 mm). Each measurement was repeated three times, and the average result was accepted as the final hydrodynamic diameters whenever all the values fluctuated within reasonable experimental errors.

Experiment 7 was for investigating the morphology of lignosulfonate 3A at the concentrations of CAC. The experiment was carried out by scanning electron microscopy (SEM-SU8010, obtained from Hitachi) with an accelerating voltage of 5 kV and a working distance of 8 mm. The analysis was done in a high vacuum, with Everhart–Thornley secondary electron (SE) detector. To prepare samples for SEM investigation, lignosulfonate 3A at 2 g/l was dissolved in ultrapure water and equilibrated for 24 h. Then the sample was added dropwise onto the surface of a polished silicon substrate and naturally dried at room temperature overnight being protected from dust in the air. The sample was thereafter coated with platinum (thickness = 5 nm) by a sputter coater (EM ACE600, obtained from Leica Vienna, Austria) before capturing SEM images. Three parallel experiments were carried out to exclude accidental errors.

Experiments 1 to 4 were carried out at Engineering Research Centre of Plant Growth Regulators, Ministry of Education in China Agricultural University in 2015, Beijing, China. Experiments 5 to 7 were carried out at Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences in 2017, Beijing, China. Experiments 1 to 3 were focused on the photodegradation and afterwards bioactivity of ABA plus lignosulfonates.

Experiments 4 to 7 were focused on the property of lignosulfonates.

2.2 Measurements

HPLC equipped with Agilent Eclipse Plus C18 column (4.6 mm×5 μm, 250 mm) (Agilent 1200, obtained from Agilent Technologies) was used to determine ABA concentrations. Methanol and water (60/40, V/V) contained 0.2 % acetic acid inside were applied as mobile phase and operated at the speed of 1 ml/min, in which temperature was set at 30 °C. UV detector wavelength was set at 260 nm. The injection volume of samples was 20 μl. ABA concentrations in samples were calculated by external standard method according to a series of ABA standard solutions.

Full wavelength scan ranging from 190 nm to 400 nm were carried out to determine absorptions of accurate amount of lignosulfonates 3A, CASA, NA, UV absorber BP-4 (2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid) and ABA in aqueous solution separately by UV/Vis spectrophotometer (UV-4802, obtained from Unico Instruments Co. Ltd). Absorptivity per unit mass was calculated from absorbance according to Lambert-Beer's Law.

UV intensity of this research and natural sunshine were detected by UVA and UVB radiationmeters (obtained from Photoelectric Instrument Factory of Beijing Normal University).

2.3 Data analysis

The bioactivity differences of ABA with ABA plus lignosulfonate 3A after being exposed to UV radiation were analyzed by One-Way ANOVA using LSD test at significant level of 0.05. Bioactivity differences of a series of lignosulfonate 3A concentrations compared to pure water were analyzed by the same statistical method as well.

194

195 **3 Results**

196 3.1 Photodegradation of ABA in the presence of lignosulfonates

197 Three types of lignosulfonates, CASA, NA and 3A significantly stabilized ABA under UV
198 radiation (Figure 3). After 8 mins' UV radiation, only 72 % ABA remained in ABA alone
199 treatment. However, all the treatments of ABA plus lignosulfonate kept at least 89 % ABA. 3A had
200 more significant effects on stabilizing ABA (degradation slope -0.005) than CASA (degradation
201 slope -0.055). Intermediate values of remained effective ABA were achieved in adding NA
202 (degradation slope -0.014).

203

204 3.2 Degradation dynamics of ABA adding with different dosages of lignosulfonate 3A

205 With adding 3A at the dose of 2 g/l to 5 g/l, more than 92 percent effective ABA remained after
206 two hour's radiation, more than 80 percent after 4 hours, and more than 67 percent after 8 hours
207 (Figure 4). Meanwhile, there was only 23 percent ABA remained in ABA alone treatment after 8
208 hours radiation. Lower level but significant values of remained effective ABA were also achieved
209 in adding 3A with doses of 0.5 g/l and 1 g/l, in which the latter showed better stabilizing effects.
210 3A at 4 g/l and 5 g/l showed no significant difference on stabilizing ABA. Though 3A at 2 g/l
211 showed significant less effect on stabilizing ABA at longer duration of radiation than at 4 g/l and 5
212 g/l, it showed nearly the same stabilizing capability in the first 4 hours' radiation. Thus, 3A at 2g/l
213 would be more convenient for further tests.

214

215 3.3 Remaining bioactivity of ABA in the presence of lignosulfonate 3A after irradiation

Shoot and root length of wheat were significantly suppressed by the diluents of 280 mg/l ABA alone or the diluents of 280 mg/l ABA plus 2000 mg/l 3A, compared to the control of pure water (Figure 5a and 5b). Shoot and root length in ABA plus 3A treatment were 20.8 % and 19.3 % shorter than that of ABA alone respectively, and there was no significant difference with the treatments of un-degraded ABA (Figure 5a and 5b). Root numbers in treatment of ABA plus 3A were 9.3 % fewer than that in ABA alone. Root numbers in ABA alone showed no significant difference with that of pure water treatment (Figure 5c). The bioactivity of un-degraded ABA with or without 3A showed no significant difference.

3.4 Impacts of lignosulfonate 3A on wheat seed germination

3A showed no significant bioactivity on wheat seed germination at the doses ranged from 1.3 mg/l to 2000 mg/l, compared to that of pure water treatment, while significant differences were found among various lignosulfonate concentrations (Figure 6a, 6b and 6c). However, 3A at the dose of 5000 mg/l suppressed 27 % shoot growth and 32 % root growth comparing to pure water treatment (Figure 6a and 6b). The root numbers in all treatments showed no significant difference with that of pure water control (Figure 6c).

3.5 Critical aggregation concentration of lignosulfonate 3A

According to the relationship between γ value and concentration (Figure 7), surface tension of lignosulfonate 3A decreased sharply when the concentration was lower than 2 g/l. However, as the concentration continued to increase, surface tension reduced slowly. So we defined concentration at 2 g/l as a critical aggregation concentration of lignosulfonate 3A in aqueous solution.

238

239 3.6 Dynamic aggregation behavior of lignosulfonate 3A in aqueous solution

240 From the intensity distribution of particle diameter for lignosulfonate 3A in aqueous solution
241 (Figure 8), there was a single peak at 0.5 g/l and 1g/l respectively, however, bimodal peaks
242 presented at 2 g/l. The smallest particle at one peak top was about 250 nm and the largest at
243 another peak top was about 5.5 μm. The intensity of particle size distribution of 3A in aqueous
244 solution decreased with increasing concentration of 3A, but the intensity and the size of 3A
245 aggregates increase. These results indicate that assemble of 3A is a gradually process.

246

247 3.7 Morphologic characterization of lignosulfonate 3A

248 From the SEM photograph (Figure 9a and 9b), aggregated structures of lignosulfonate 3A at the
249 concentration of 2 g/l were observed. The aggregated structures were within the diameters of
250 about 500 nm to about 5μm and the larger ones appeared to be hollow, as deep grey color in the
251 middle of the aggregated structures was observed (Figure 9b). Solid aggregated structures would
252 show the same pale white color with the exterior, but if the structures are hollow, the middle
253 would be collapsed under the strong voltage of SEM. This resulted in deep grey color in the center
254 of the aggregated structures.

255

256 **4 Discussions and conclusions**

257 By adding lignosulfonates, ABA degradation under UV light was significant reduced.
258 Lignosulfonate greatly kept the bioactivity of ABA after exposed to UV radiation. Lignosulfonate
259 itself had no significant bioactivity. Adding lignosulfonate kept 67 percent ABA remained after 8

hours exposing to UV irradiation, while only 23 percent ABA remained in ABA alone control. The efficiency of ABA was increased nearly 3 times by adding lignosulfonate.

It is well known that UV bands of field sunshine mainly range from 290 nm to 400 nm (*55*). At this range, ABA has some absorption in UVB (290 nm ~ 320 nm) but very little in UVA (320 nm ~ 400 nm) (*56*) (Figure 10). Thus, UVB takes a great role on ABA bioactivity reduction in the field. Lignosulfonates absorb UV radiation at most of UV region due to many chromophores it contained. And the complex structure of lignosulfonate, which is widely accepted as a kind of three-dimensional cross-linked biopolymer (*57*), can effectively dissipate the energy associated with the absorption of UV light internally thereby preventing transfer to other proximate actives. Though absorption of lignosulfonate per unit mass is not greater than that of ABA (Figure 10), especially, in UVB region, increased dosage of lignosulfonate could show great UVB absorption. Our experiments proved that lignosulfonate at proper high mass dosage strongly competed UVB absorption, compared to that of ABA. Besides, the UV radiation applied in this research mainly ranged from UVB to UVA (S1). UVB intensity applied in this study (2730 $\mu W/cm^2$) was much higher than under natural sunshine (263 $\mu W/cm^2$). Therefore, laboratory results in this study that ABA photodegradation could be greatly reduced by competitive UV absorption of lignosulfonate can be applicable in field situation.

Lignosulfonate with different molecular weight and sulfonation degree showed different stabilizing effects on ABA degradation under UV radiation. Lignosulfonate with higher molecular weight may have more aryl ring structural units (*58*), which strengthens and shifts the UV absorption of lignosulfonate to longer wavelength and thereby absorbs more UV damage from ABA around. Lignosulfonate with lower sulfonation degree may have more α-O-4 ether bonds not

being replaced by sulfonic acid groups (*57*), which also enhances and induces red shift and finally help improve UV protection effects. 3A with low degree of sulfonation and high molecular weight, thus, showed most preferable photostabilizing effect on ABA degradation.

Absorptivity of lignosulfonate per unit mass is much lower than that of UV absorbers such as 2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid (BP-4) (Figure 10), therefore, theoretically, much higher dosages of lignosulfonate should be applied to stabilize ABA comparing to that of UV absorber. According to our measurements in this study and in the former researches (*37*), we found 3A at 500 mg/l showed no significant difference with that of BP-4 at 200 mg/l on stabilizing ABA under UV radiation (Figure 11). However, adding 2000 mg/l 3A had a much higher stabilizing effect on ABA than adding BP-4. In consideration of potential impacts on human health and the environment (*38-43*), caution is needed to increase the dosage of BP-4 applied on ABA in order to improve its stabilizing effects. Lignosulfonate, however, is sulfonated natural lignin, proved safety to human beings (*49, 59*) and degradable in the environment (*60*). Lignosulfonate in aqueous solution is quite stable even under strong and short wavelength UV radiations (*61-63*). Properly increasing dosages of lignosulfonate was proved little impacts on plant growth by our experiments. Thus, lignosulfonate is more efficient and eco-friendly to stabilizing ABA under UV radiation even applied at high dosage.

Lignosulfonate 3A at 2 g/l (CAC) showed similar photostabilizing effects with that of 3A at higher concentration (4 g/l or 5 g/l). There is no significantly increased effect on ABA photostabilizing by further increasing lignosulfonate 3A above CAC, which indicates there might be other factors that also protect ABA from UV damage except for competitive UV absorption. One factor might be the micelles that lignosulfonate formed. It is well known that micelles were

formed by surfactants above critical micelle concentration. A typical micelle is an aggregate with the hydrophilic "head" regions in contact with surrounding solvent, sequestering the hydrophobic single-tail regions in the micelle center. Though lignosulfonate has amphiphilic activity and could form micelles, the lignosulfonate molecules cannot aggregate into hollow micelles by their hydrophobic sites as conventional linear molecule surfactants, as the hydrophobic base and hydrophilic groups in lignosulfonate molecules are not totally separated from one another and the hydrophilic groups are scattered in the whole molecule (Figure 12) (*64*). According to our research and other studies (*46, 64*), hollow aggregates are the main form of the lignosulfonate at the dosages of above CAC in solution. Moreover, sulfonic groups and some phenolic hydroxyl groups constructed the surface of lignosulfonate aggregates. And the core of the aggregates is formed by hydrophobic chains, which is loose and contains many weakly ionized groups such as carboxyls and phenolic hydroxyl groups (Figure 12) (*46*). ABA has hydroxyl, conjugated carboxyl and ketene groups and is slightly dissolved in water (3 g/l) and strongly dissolves in methanol, acetone and ethyl acetate (above 90 g/l) (*65*), thus it showed relative weak polarity which makes it tend to presence in the core of lignosulfonate aggregates through hydrogen-bonding and intermolecular van der Waals forces (Figure 12). This explains the similar stabilizing effects of lignosulfonate 3A at dosages above CAC. Therefore, we concluded that the formation of hollow aggregates might be the other important factor that helped lignosulfonate protect ABA from UV damage. CAC found in this study can be applied as one of the reference indexes for adding lignosulfonate into ABA.

Absorbance of agrichemicals by plant stems and leaves mainly takes place in the first four hours after their application. According to our former measurement in natural conditions of winter time, the applied ABA (540 mg/l) lost more than 50 % within 4 hours and even worse during other

326 seasons due to much higher UV radiation. By adding lignosulfonate, the loss of ABA bioactivity
327 can be reduced to less than 20 % and even much lower. Although our previous studies (*37*) proved
328 UV absorber BP-4 had a good photostabilizing effect on ABA, however, the simple small
329 molecule of BP-4 might loss its stabilizing capability on ABA due to the rain. Lignosulfonate has
330 amphiphilic groups and plant leave surface is always hydrophobic or hydrophilic, which could
331 help ABA well adhere on the leaves and thereby increases its effects on ABA stabilization.
332 Therefore, the usage of surfactant could be thereafter reduced and the limitation of using BP-4 can
333 be relieved.

334 In the future studies, appropriate time and dosage of lignosulfonate added into ABA application
335 are still required, especially, by more experiments at field conditions. In the field, the bioactivity
336 of ABA plus lignosulfonate in the sunny day might be different from that of in the cloudy due to
337 different degrees of degradation, so the effect and suitable doses of lignosulfonate should be
338 testified at both situations in the future. The effects of ABA plus lignosulfonate on growth,
339 development and yields of plant in relation to optimal applying time and dosage should be
340 determined at field condition. Moreover, the effects of new ABA products with adding
341 lignosulfonate on prolonging florescence, promoting fruit coloring rate and improving resistance
342 to drought stress is necessary to be explored, especially under natural sunshine.

343 In conclusion, lignosulfonate greatly reduced ABA photodegradation. Lignosulfonate 3A
344 addition significantly prevented ABA from UV damage and thereafter greatly kept ABA
345 bioactivity after exposed to UV radiation. Besides, lignosulfonate showed no significant impacts
346 on plant growth at suitable dosage. Consequently, lignosulfonate could be a safe and high
347 efficiency agent to photostabilize ABA. The usage of surfactants could also be reduced due to the

surface activities of lignosulfonate. And waste liquor of sulfite pulp mills can be reduced and reused. Pretty low cost would be made by applying lignosulfonate to prepare novel photostable formulations of ABA or alternatively to be applied tank-mixed in the field, compare to the application of UV absorbers. Thereafter, the ABA efficiency would be significantly promoted and crop yield and quality would be improved. This research could be widely used in the application of UV-sensitive and water-soluble agrichemicals and help to reduce environmental impact of applying plant growth regulators.

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Figure captions

Figure 1 Structure and photoisomerization of ABA to *trans*-ABA.

Figure 2 Sulfonation process of lignin in sulfite pulping. Lignin in the group sites of the structure represents for propagated lignin structure. This figure was modified from Lauten et al (*57*).

Figure 3 Photodegradation dynamics of ABA in the presence of varieties of lignosulfonate. ABA was applied at 5 mg/l. Lignosulfonate 3A, CASA and NA were all applied at 500 mg/l. Error bars are s. e. (standard error) of three replicates.

Figure 4 Dynamics of ABA (280 mg/l) photodegradation in aqueous solution affected by adding different doses of lignosulfonate 3A. ABA was applied at 280 mg/l. Lignosulfonate 3A was applied at 500 mg/l, 1000 mg/l, 2000 mg/l, 4000 mg/l and 5000 mg/l. lingo. is the abbreviation for lignosulfonate 3A. Error bars are s. e. of three replicates.

Figure 5 Bioactivity of ABA in the presence of lignosulfonate 3A after irradiation on the growth of wheat (JIMAI 22). ABA (280 mg/l) with or without 3A (2000 mg/l) were exposed to UV radiation for 2 h and then diluted for 300 times to compare their bioactivity on wheat shoot and root growth. Error bars are s. e. of five replicates. Within each figure, treatments with same letter are not significantly different at a=0.05. un-deg. is the abbreviation for un-degraded; p-deg. is the abbreviation for partially-degraded.

Figure 6 Impacts of lignosulfonate 3A on the growth of wheat (JIMAI 22). 3A is applied at 1.3 mg/l, 6.7 mg/l, 33.3 mg/l, 500 mg/l, 1000 mg/l, 2000 mg/l and 5000 mg/l. Error bars are s. e. of five replicates. Within each figure, treatments with same letter are not significantly different at a=0.05.

Figure 7 Surface tension of lignosulfonate 3A as a function of 3A concentration at 25 °C. CAC is the abbreviation for critical aggregation concentration. γ represents for surface tension.

551 **Figure 8** Influence of concentration on the particles size distribution of lignosulfonate 3A at
552 25 °C.

553 **Figure 9** SEM micrography of lignosulfonate 3A on polished silicon substrate. Lignosulfonate
554 was applied at the concentration of 2 g/l. a and b represent for micrograph captured at different
555 magnification.

556 **Figure 10** Absorption spectra per unit mass of ABA, lignosulfonate 3A, CASA and NA, and UV
557 absorber BP-4 in aqueous solution. Gray and light gray shadows indicate UVB (290-320 nm) and
558 UVA (320-400 nm) region in natural UV radiation.

559 **Figure 11** Comparison effects of lignosulfonate 3A and UV absorber BP-4 on stabilizing ABA
560 under UV radiation. ABA was applied at 280 mg/l. Error bars are s. e. of three replicates.

561 **Figure 12** The formation of lignosulfonate aggregate and its interaction with ABA.
562

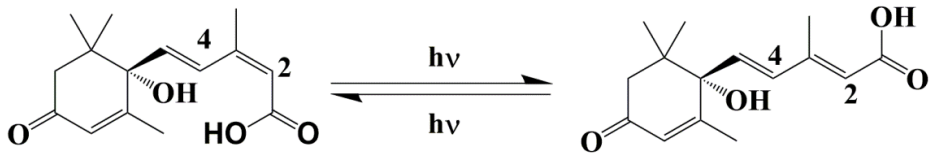
563 **Tables**

564 Table 1 Physico-chemical properties of three commercialized lignosulfonates

Name	Molecular weight	Na (%)	Ca (%)	Sulfonation degree
3A	> 50000	8.5	0.02	0.48
NA	20000 ~ 50000	9	0.3	0.7
CASA	< 20000	< 0.1	5	0.7

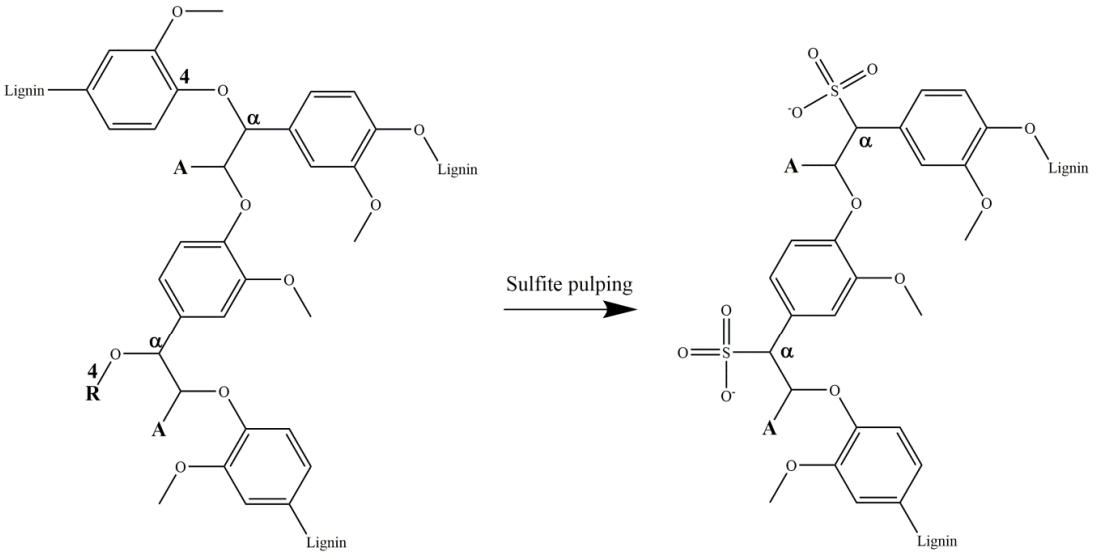
565

Figure graphics



ABA ***trans*-ABA**

Figure 1

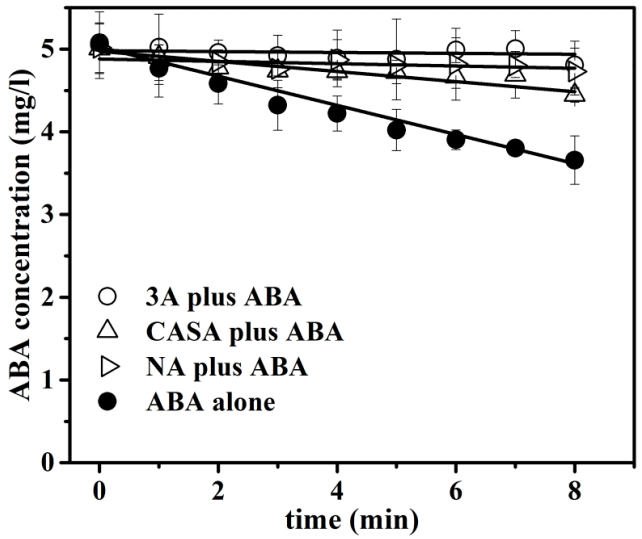


R=H, sugar, lignin
A=Varous groups, e.g. CH_2OH, CHO, COOH

570

571 Figure 2

572



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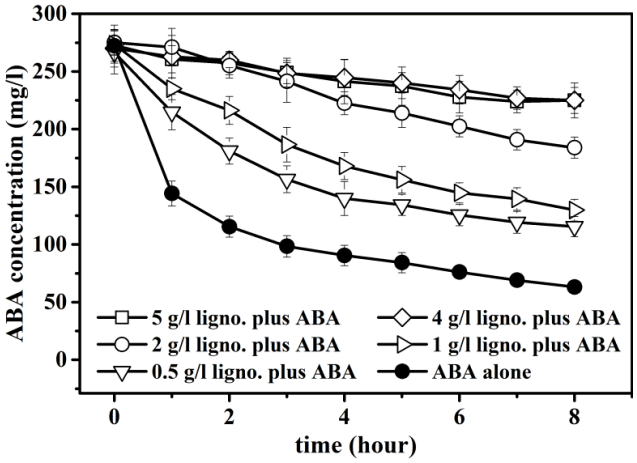
574 Figure 3



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576 Figure 4

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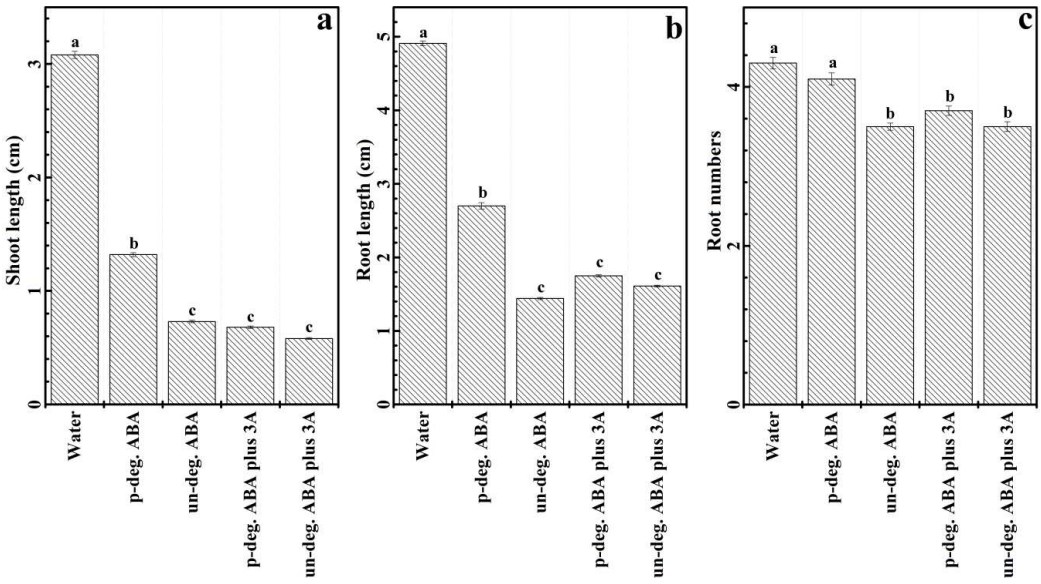

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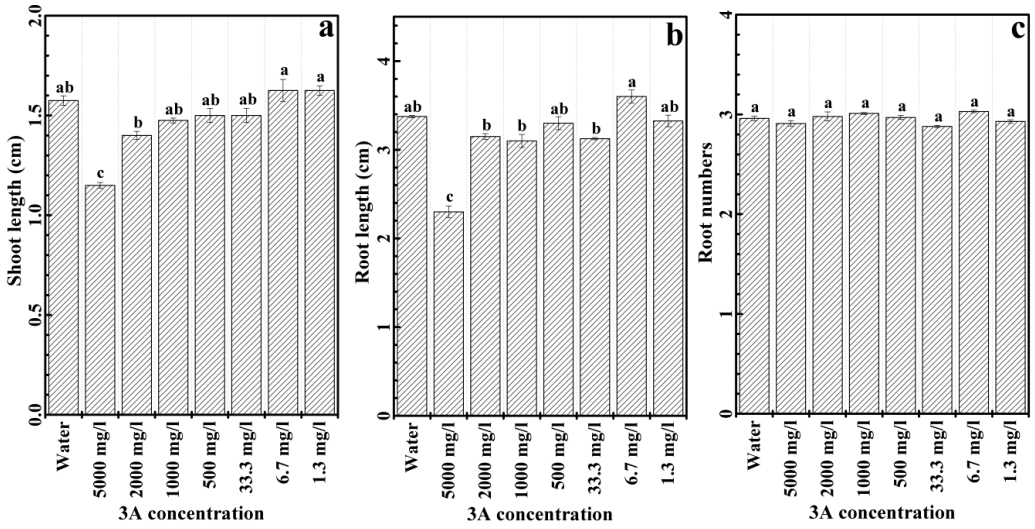
579 Figure 5



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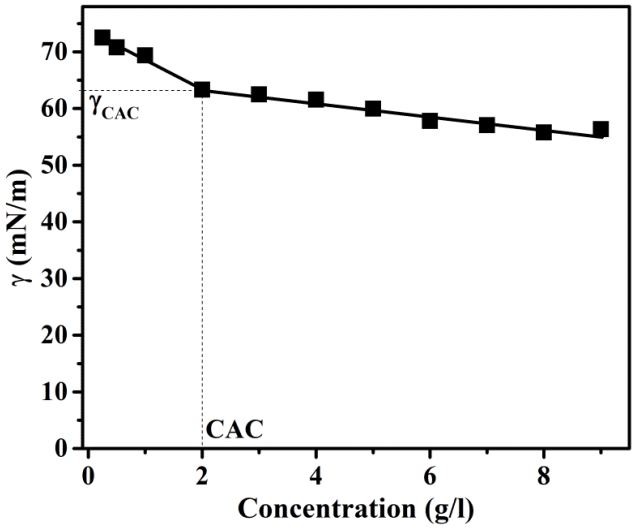
581 Figure 6



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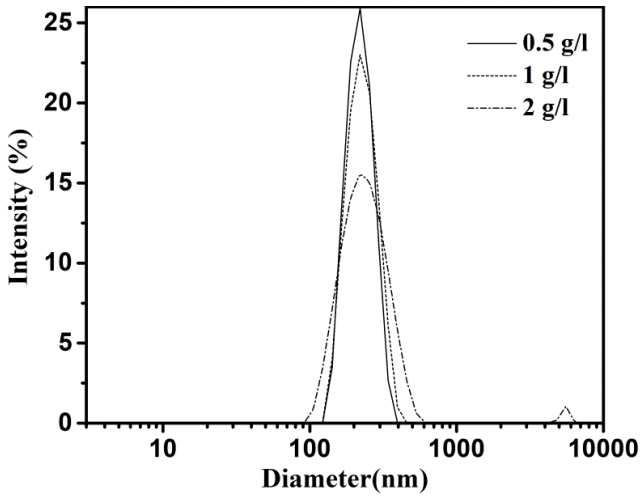
583 Figure 7



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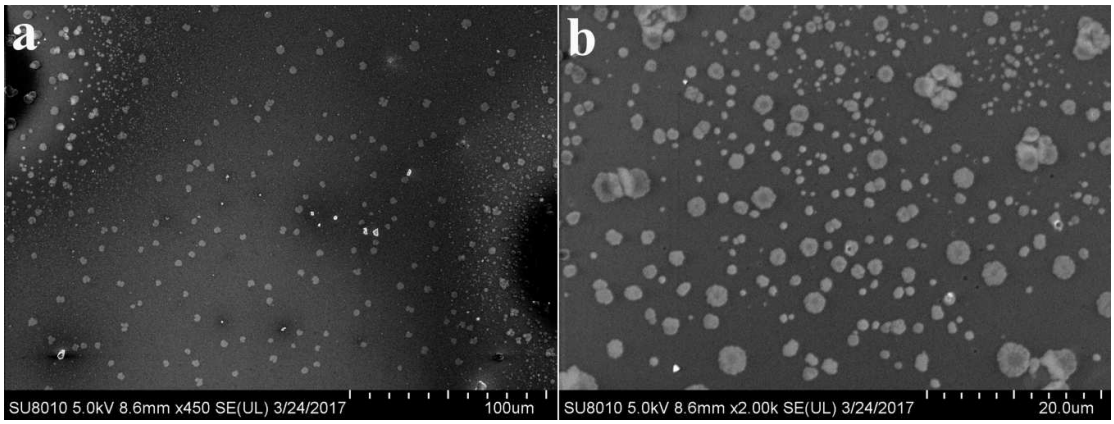
585 Figure 8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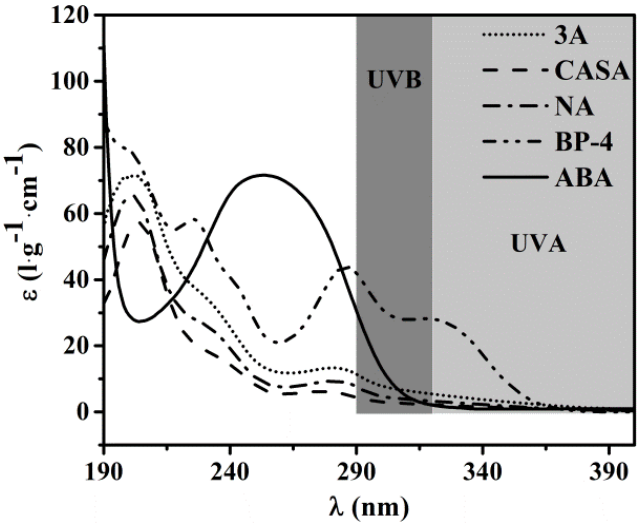
100um
b
SU8010 5.0kV 8.6mm x2.00k SE(UL) 3/24/2017
20.0um

Figure 9



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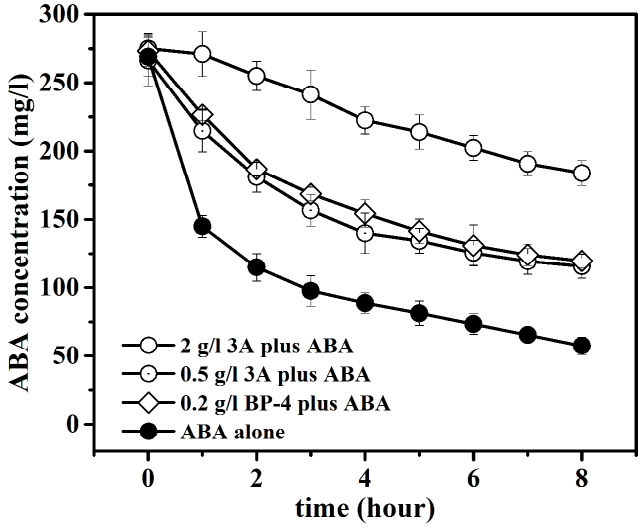
589 Figure 10



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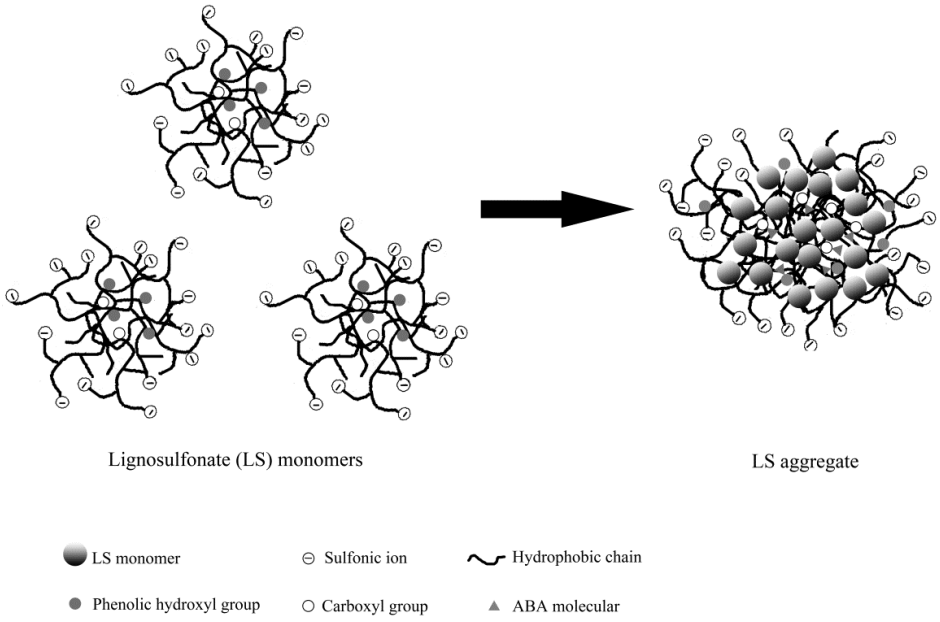
591 Figure 11

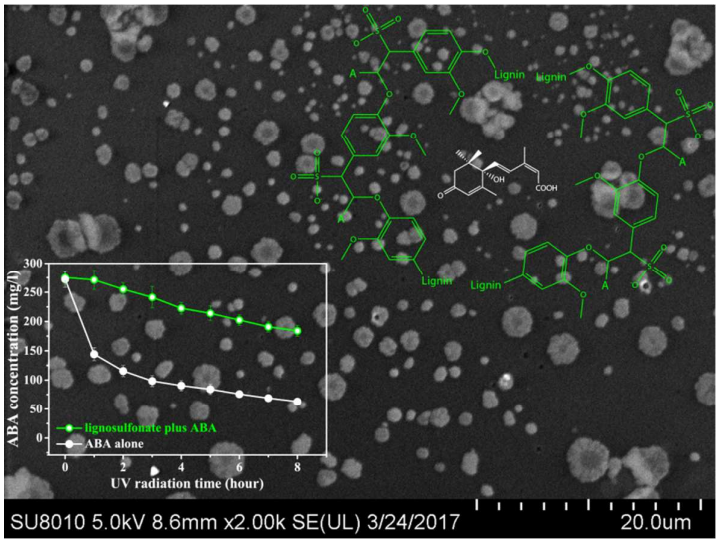


592

593 Figure 12

594

595 **Graphic for table of contents**



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