

Influence of simulated acid rain on the physiological response of flowering Chinese cabbage and variation of soil nutrients

SHUO MA^{1,2}, WENKANG CHEN^{1,2}, JIAEN ZHANG^{1,2*}, HONG SHEN^{1,2*}

¹College of Natural Resources and Environment, South China Agricultural University, Guangzhou, P.R. China

²Guangdong Provincial Key Laboratory of Eco-Circular Agriculture, Guangzhou, P.R. China

*Corresponding authors: jeanzh@scau.edu.cn; hshen@scau.edu.cn

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Abstract: Flowering Chinese cabbages are widely planted in the south of China and often exposed to acid rain. However, the effect of acid rain on the growth of flowering Chinese cabbage is unclear. In this study, we investigated the influence of simulated acid rain (SAR) on plant height, soil-plant analysis development (SPAD) value (an index for chlorophyll content), proline, malondialdehyde (MDA), antioxidant enzyme activities, nitrogen (N), phosphorus (P), or potassium (K) uptake and variation of soil nutrients. Our results showed that SAR at pH 5.5 did not damage plant development because growth characteristics, photosynthesis, and superoxide dismutase and peroxidase activities did not change obviously at this pH compared to those at pH 7.0. However, 2- to 7-time of SAR exposure at pH 4.5 and pH 3.5 led to the increases of antioxidant enzyme activities, MDA and proline contents, and the decreases of leaf SPAD value and root activity. Nutrient analysis indicated that spraying 4 to 7 times of SAR at pH 3.5 reduced the uptake of N, P and K of flowering Chinese cabbage significantly. In addition, treatment with SAR at pH 3.5 decreased the pH value of the surface soil and the contents of alkaline-hydrolytic N and readily available K but increased that of readily available P in the surface soil by 8.5% to 14.9%. Taken together, our results indicated that SAR at pH 3.5 influenced the antioxidant enzyme system and the contents of soil nutrients, caused metabolic disorders and ultimately restricted the development and growth of flowering Chinese cabbages.

Keywords: atmospheric pollution; acidification; nutrient availability; leafy vegetable; abiotic stress; phosphorus fractions

The acid rain problem caused by industrial production and human activities has become more and more serious, and China has become the third-largest acid rain pollution area after Europe and North America. Survey data show that the distribution area of acid rain in China accounts for 5.5% to 6.4% of the total land area in China, mainly in the south of the Yangtze river and the east of the Yunnan-Guizhou Plateau. As the basic unit of the terrestrial ecosystem, plants and soil are extremely vulnerable to acid rain.

Acid rain causes direct damage to plants by directly contacting leaves. Many studies have shown that acid rain can easily cause excessive accumulation of reactive oxygen species (ROS) in plant cells, leading to the

increases of malondialdehyde content and membrane permeability, which resulted in structural damage to cells and inhibited the normal growth of plant shoots and roots (Yu et al. 2015, Hu et al. 2016, Du et al. 2017). Severe acid rain decreases the stability of thylakoids, and the synthesis of chlorophyll, thereby reduced the efficiency of light energy conversion and inhibited plant growth (Yu et al. 2015). To cope with acid rain stress, plants have evolved a series of tolerant mechanism. For example, the increases of antioxidant enzyme activities and antioxidant substance contents of tomato seedlings contribute to protect themselves from toxic reactive oxygen species (Debnath et al. 2018).

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Soil is an important part of the terrestrial ecosystem and is easily polluted by acid rain. Acid rain can cause the acidification of surface soils, accelerate the loss of soil exchangeable ions, and affect soil nutrients and fertility (Kim et al. 2010, Li et al. 2019). The deficiency of nutrients (such as calcium, magnesium, etc.) indirectly affects the growth of plants (Mishima et al. 2013). Meanwhile, soil that is severely damaged by acid rain will release a large number of heavy metal ions, which will restrain the normal growth of plants (Kim et al. 2010). In addition, acid rain can also change the micro-environment of the soil, inhibit the activities of soil microorganisms and enzymes related to soil nutrient cycling, thereby affect the conversion efficiency of soil nutrients, including nitrogen (N), phosphorus (P), and sulfur (S) (Wang et al. 2018).

Flowering Chinese cabbage (*Brassica campestris* L. ssp. *Chinensis* var. *utilis* Tsen et Lee) is an important leafy vegetable planted in China, and its planting area is the largest among the leafy vegetables in South China. Flowering Chinese cabbages are often cultivated in outdoor fields, and the production process is vulnerable to natural disasters. However, acid rain, as one of the common natural disasters in South China, is particularly harmful to outdoor cabbage cultivation. At present, many studies on the stress of cabbage in terms of adversity stress focus on high or low-temperature stress, heavy metal accumulation, antibiotic substances accumulation, or other fields (Hajiboland and Amirzad 2010, Kim et al. 2010). However, the studies on the physiological characteristics of flowering Chinese cabbage in responses to acid rain stress are still scarce. In this study, we investigate the physiological responses of flowering Chinese cabbage and variation of soil nutrients in the rhizosphere under simulated acid rain conditions. The objective of this study is to explore the damage mechanism of acid rain to flowering Chinese cabbages from the perspectives of both direct oxidative damages and indirect hindering effect of soil fertility and nutrient uptake.

MATERIAL AND METHODS

Experimental site. The experiment was performed at the greenhouse with plastic film-roof and gauze walls in the College of Natural Resources and Environment, South China Agricultural University (113°21'E, 23°9'N), Guangzhou, P. R. China, from June 2018 to December 2019. This area has

a typical subtropical monsoon climate: annual average sunshine time, 1 607 h; annual average temperature, around 21.9 °C; annual average rainfall, 1 800.5 mm; annual rainy days, around 150 days (Pan 2019).

Plant materials and treatments. In this experiment, the cultivar of flowering Chinese cabbage was cv. Youqing 40-day. Cabbage seeds were germinated in a mixed matrix containing vermiculite, peat and perlite (1:2:1, v/v). When the third true leaf was fully expanded, the seedlings were transplanted to soil in pots (a cylinder with 16-cm diameter and 18-cm height). Each pot contained 5 kg soil, and 2 seedlings were planted in a pot. The soil used in this study were collected from the soil layer in depth from 0 cm to 20 cm in the local vegetable garden. The chemical properties of the soil were as follows: organic matter – 24.8 g/kg (equivalent to 14.2 g/kg of dichromate-oxidizable carbon); total N – 1.23 g/kg; total P – 452 mg/kg; total potassium (K) – 320 mg/kg; alkaline-hydrolytic N – 68.3 mg/kg; readily available P – 45.8 mg/kg; readily available K – 173.3 mg/kg; available S – 95.9 mg/kg; pH (aqueous extraction, ratio of soil and water was 1:2.5) – 6.59. Growth conditions for cabbage plant after transplantation were: temperature 25 °C/18 °C (day/night), relative humidity 70–80%, photoperiod 16 h/8 h (day/night).

The simulated acid rain (SAR) solution was prepared by sulfuric acid and nitric acid. Firstly, the acid stock solution of pH 1.0 was prepared according to the ratio of an equivalent concentration of SO_4^{2-} : NO_3^- = 4:1 based on the study of Qin et al. (2006) on acid rain in Guangzhou. Then the solution was diluted with tap water to pH 3.5, pH 4.5, pH 5.5 and pH 7.0 (the control: CK), which covered the common range of acid rain in this region (3.5–4.8) (Dai et al. 2013, Shu et al. 2019, Du et al. 2020) and representative normal rain. Treatments were performed 4 days after transplanting. The cabbage seedlings were sprayed with SAR once every 3 days. The ages of seedlings were 20, 23, 26, 29, 32, 35, or 38 days, respectively, when they were subjected to the first to the seventh exposure. The spraying time was 4:00 p.m. The amount of each spray was 96.6 mL. During the period of SAR treatment, a proper amount of tap water was poured to maintain soil moisture. On the second day after each spray of SAR, the third to seventh leaves from the bottom to the top were collected for further analysis.

Determination of plant height, dry weight, SPAD value and root activity. The height of flowering

Chinese cabbage was measured by a ruler with millimeter graduations. The dry weight of flowering Chinese cabbage was measured by an electronic balance with a precision of 0.1 mg. The soil-plant analysis development (SPAD) value of the third leaf from the bottom was measured by a chlorophyll meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan). Root activity was measured by the α -naphthylamine oxidation method, and the amount of oxidized α -naphthylamine by 1 g of fresh roots after 1-h shaking was used to express root activity (Shu et al. 2019).

Determination of MDA and proline and antioxidant enzyme activity. The content of malondialdehyde (MDA) was determined by the thiobarbituric acid method, expressed as the molar concentration of MDA on a fresh mass tissue basis. The content of proline was determined by the acidic ninhydrin method, expressed as the mass of proline in fresh tissue mass basis. The activity of superoxide dismutase (SOD) was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazole (NBT). Guaiacol was used as the substrate to evaluate the activity of peroxidase (POD). The details of enzyme activity assay was described by Li (2000).

N, P and K uptake in cabbages. Firstly, plant samples were dried, ground and sieved. Then 0.1 g of powder sample was weighed and digested with sulfuric acid and hydrogen peroxide. The digestion solution was diluted for other N, P, K measurements. N, P, and K uptake of flowering Chinese cabbage were determined by Kjeldahl's distillation – absorption – titration method, molybdenum blue spectrophotometry, and atomic absorption spectrometry, respectively. The details of digestion and subsequent determination were described by Bao (2000).

Determination of soil chemical properties. After SAR treatment, soil samples were collected at the depths of 0, 4, 8, 12, and 16 cm in the rhizosphere of flowering Chinese cabbage. After natural air drying, soil samples were crushed, mixed and ground, and passed through a 1-mm sieve. Soil pH value was determined by the aqueous solution extraction method in the ratio of 1:2.5 (*w/v*). Soil alkaline-hydrolytic N was determined by the alkaline diffusion method. Soil readily available P was determined by hydrochloric acid-ammonium fluoride extraction combined with molybdenum blue spectrophotometry method. The readily available K was determined by ammonium acetate extraction combined with flame atomic absorption spectrophotometry. The composition of inorganic phosphorus in soil was determined by the

modified Chang-Jackson extraction method. The details of the above extraction and determination were based on the instruction of Bao (2000).

Statistical analysis. The data in this study were analysed by one-way analysis of variance (ANOVA) and general linear models with SPSS 25.0 (Chicago, USA).

RESULTS AND DISCUSSION

Effect of simulated acid rain on the growth of flowering Chinese cabbage. Previous studies indicated that acid rain influences not only the morphology but also the physiological processes of plants (Debnath et al. 2018). The direct effect of acid rain on plants is damaged in chloroplasts, and leaf chlorophyll content decrease (Du et al. 2017). In this study, we investigated the changes in plant height, biomass, leaf SPAD value and root activity of flowering Chinese cabbage under acid rain stress (Figure 1) and found that the plant height, biomass, leaf SPAD value and root activity of flowering Chinese cabbage showed an upward trend in the vegetative growth phase with the extension of the growth period under the acid rain stress. After spraying twice, treatment with SAR at pH 3.5, pH 4.5, and pH 5.5 reduced the plant height of flowering Chinese cabbage by 26.06, 24.41, and 10.48%, respectively, compared with the control. Meanwhile, the root activity with pH 3.5 and pH 4.5 SAR treatments was decreased by 74.51% and 34.90%. Spraying pH 3.5 and pH 4.5 SAR for 3 times reduced the SPAD values of flowering Chinese cabbage leaves by 10.91% and 8.92%. Spraying 4 times of pH 3.5 and pH 4.5 SAR reduced the dry weight of flowering Chinese cabbage by 46.08% and 43.14%, respectively. Acid rain stress shortens plant heights and reduces root activity (Wang et al. 2018), and damages the structure of plant leaves, resulting in a significant decrease in chlorophyll content, photosynthesis, and biomass (Hu et al. 2016, Du et al. 2017). Our results supported the above views that spraying 2 to 7 times of pH 3.5 and pH 4.5 SAR could reduce leaf SPAD value and root activity and thus inhibit the growth of flowering Chinese cabbages (Figure 1).

Effect of simulated acid rain on MDA, proline and antioxidant enzyme activity. As the final product of membrane lipid peroxidation, MDA content can indicate the degree of membrane lipid peroxidation (Debnath et al. 2018). Exposure to acid rain treatments with different pH, MDA and proline contents of flowering Chinese cabbage leaves showed different responses (Figure 2). The content of MDA did not

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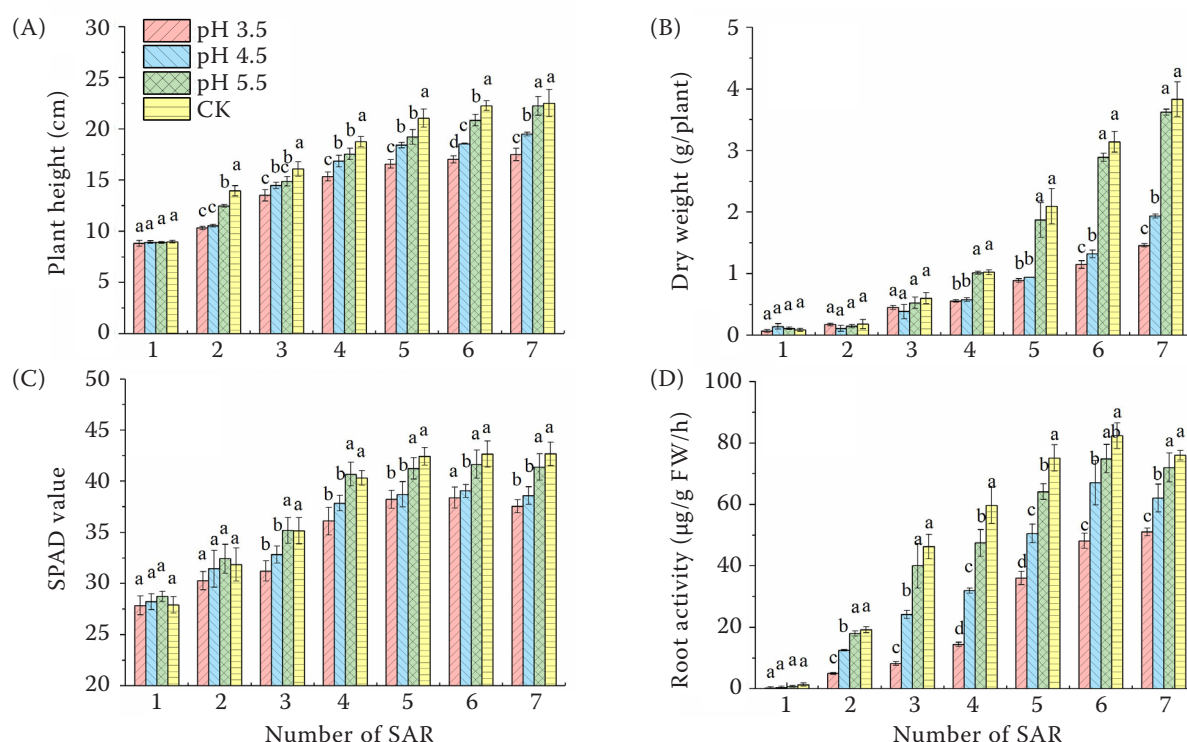


Figure 1. Variation of growth of flowering Chinese cabbage under simulated acid rain (SAR) stress. (A) plant height; (B) dry weight; (C) soil-plant analysis development (SPAD) value, and (D) root activity. Vertical bars indicated the standard error. Different letters with the same spray time indicated significant differences at $P < 0.05$ according to the Duncan's test; FW – fresh weight; CK – control

vary after spraying once, while the content of proline increased after spraying once of pH 3.5 SAR significantly. The contents of MDA and proline increased obviously after 2, 3, 4, or 5 times spray. MDA contents after pH 3.5 and pH 4.5 SAR treatment with 3- to 6-times spray were increased by 26.09% to 58.18% and 25.04% to 35.27%, respectively, compared with the control. But no difference of MDA was observed between the control and SAR treatment at pH 5.5 with 7 times spray (Figure 2A). The content of proline, a major osmoprotectant in plants, is often increased by abiotic stress, such as drought and salt. Figure 2B indicated that acid rain increased the contents of proline, and proline contents among different SAR treatments showed a similar, varying pattern. The proline contents of flowering Chinese cabbage with different SAR treatments presented the order of pH 3.5 > pH 4.5 > pH 5.5 ≈ CK. Our results agreed with the previous report (Ren et al. 2018) that acid rain treatments produce higher proline content, and this phenomenon might be due to cell water status alteration (Shu et al. 2019).

Ren et al. (2018) indicated superoxide dismutase plays an important role in the protection and regu-

lation of plant growth by declining ROS, which are produced with the large amount under acid rain stress, levels in plant cells. The results of Figure 2C indicated that the activity of SOD showed an overall upward trend with the elongation of the growth period of flowering Chinese cabbage. Spraying SAR at pH 3.5 for 1 to 3 times increased SOD activity in the leaves of flowering Chinese cabbage by 52.16% to 65.66% compared with the control, while spraying pH 4.5 and pH 5.5 SAR did not influence SOD activity of flowering Chinese cabbage significantly. It's noteworthy that spraying SAR at pH 3.5 and pH 4.5 with 4 to 7 times elevated the SOD activity significantly, which were 1.60 to 1.94 times and 1.26 to 1.64 times of the control, respectively. Being exposed to acid rain stress, higher activity of SOD can alleviate the damage caused by ROS accumulation in rice (Ren et al. 2018). Our results suggested that high activity of SOD could alleviate SAR-induced injuries of flowering Chinese cabbage.

Peroxidase belongs to the member of protective enzymes in plants, and it plays a key role in the plant enzymatic defense system by catalysing hydrogen peroxide and participating in the physiological

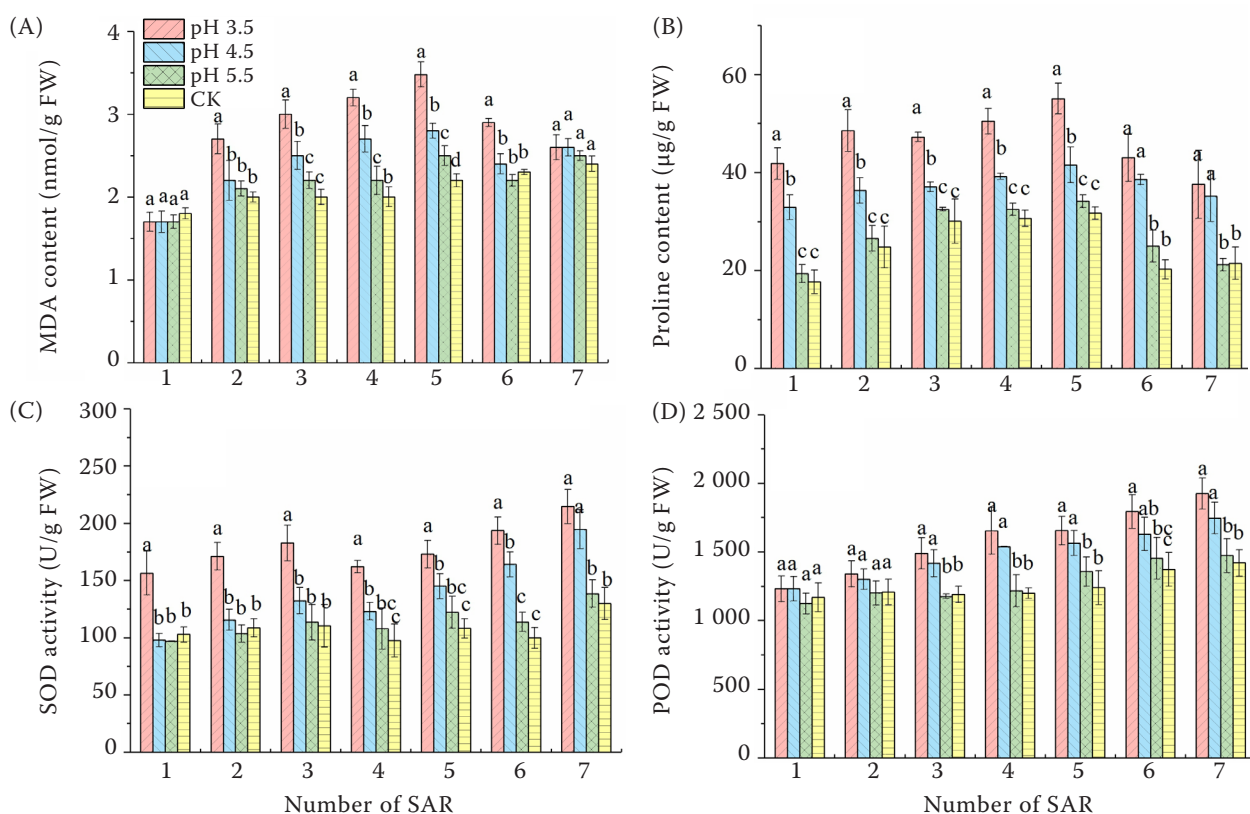


Figure 2. Variation of leaf malondialdehyde (MDA), proline and antioxidant enzyme activity of flowering Chinese cabbage under simulated acid rain (SAR) stress. (A) MDA content; (B) proline content; (C) the activity of superoxide dismutase (SOD), and (D) the activity of peroxidase (POD). Vertical bars indicated the standard error. Different letters with the same spray time indicated significant differences at $P < 0.05$ according to the Duncan's test. FW – fresh weight; CK – control

metabolism of organisms. As shown in Figure 2D, the POD activity of flowering Chinese cabbage leaves kept an increasing trend during the life cycle of the plant. Spraying SAR once or twice did not affect the activity of POD obviously, while spraying 3 to 7 times SAR at pH 3.5 and pH 4.5 elevated POD activity by 1.26 to 1.38 times and 1.15 to 1.28 times of the control. The activities of POD among three SAR treatments followed the order of pH 3.5 > pH 4.5 > pH 5.5. Since the damage of leaves became gradually severe with decreasing pH values of acid rain that the leaves were exposed to (Figure 2A, B) due to ROS accumulation (Cao 2010), it is common that antioxidant enzymes including SOD and POD were activated to scavenge ROS (Liu et al. 2015, Shu et al. 2019). Ren et al. (2018) and Guo et al. (2019) suggested that the antioxidant enzyme activities kept rising with increasing concentrations of ROS when the pH of acid rain was higher than 3.5. Similar significant results of POD activity were also observed in rice seedlings subjected to acid rain treatment (Wang et al. 2019).

Effect of simulated acid rain on N, P, K uptake in flowering Chinese cabbage. Nitrogen, phosphorus and potassium are three essential nutrient elements for plant growth, which play an important role in the process of plant growth, and their contents vary with plant species, growth stage and environmental conditions (Mishima et al. 2013). In this study, we measured N, P, K uptake in flowering Chinese cabbage in response to different SAR treatments. As shown in Figure 3A, N uptake of flowering Chinese cabbage increased when plants became mature. However, N uptake was reduced greatly after SAR treatment. N uptake of flowering Chinese cabbage with spraying pH 3.5 and pH 4.5 SAR for 7 times was 36.87% and 50.15% of those control. Zhang et al. (2017) reported that pH 3.5 acid rain treatment reduces the N content by 33.56% in the roots of rice. Similarly, Hu et al. (2016) found that N content in rice seedlings decreases with the decrease of simulated acid rain pH value. Our results supported the above view that acid rain was adverse to plant N absorption.

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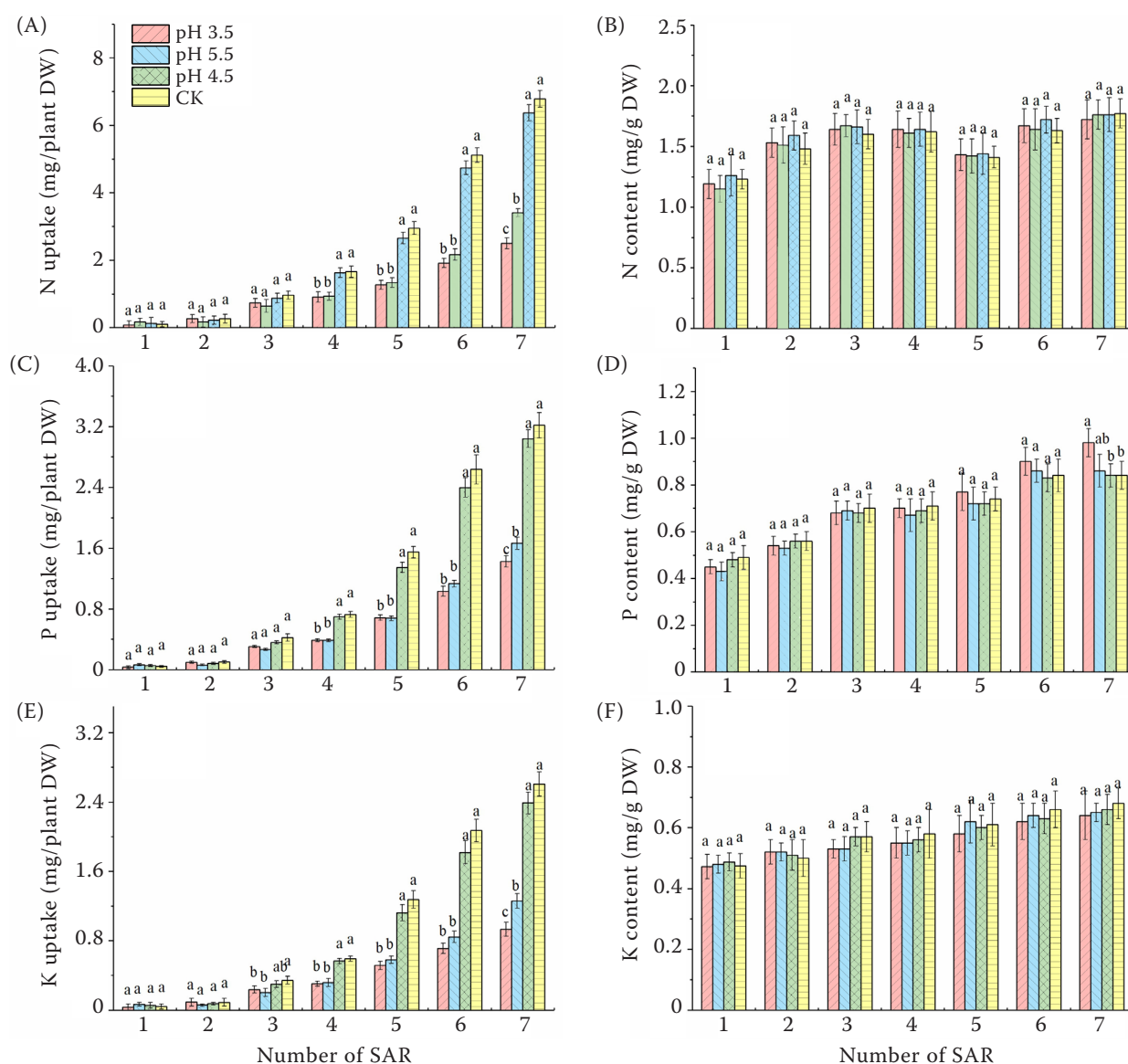


Figure 3. Nutrient uptake and content of flowering Chinese cabbage under simulated acid rain (SAR) stress. (A) nitrogen uptake; (B) N content; (C) phosphorus uptake; (D) P content; (E) potassium uptake; (F) K content. Vertical bars indicate the standard error. Different letters with the same number of spray time indicated significant differences at $P < 0.05$ according to the Duncan's test; DW – dry weight; CK – control

P uptake of flowering Chinese cabbage showed a similar trend to N uptake when exposed to SAR treatments. Flowering Chinese cabbage P uptake was decreased by 27.76% to 55.75% compared with the control when exposed to SAR at pH 3.5 with 3 to 7 times (Figure 3C). Notably, the P concentration of cabbage with 6-time spraying SAR at pH 3.5 was increased by 7.14% compared to the control, but the difference was not statistically significant (Figure 3D). However, spraying SAR at pH 3.5 for 7 times increased P concentration by 16.67% obviously. Consistent with our research results, P concentration in tea leaves

under acid rain stress at pH 3.5 is higher than that of the control (Hu et al. 2016).

K uptake of cabbage increased slightly as the growth stage went forward (Figure 3E). SAR treatment decreased the K uptake of flowering Chinese cabbage. K uptake of cabbage treated with pH 3.5 and pH 4.5 SAR was decreased by 31.13% to 64.30% and 40.74% to 51.75%, respectively, compared with the control (Figure 3F). Hu et al. (2016) reported that treatment with simulated acid rain at pH 3.5 and pH 2.5 reduces the K content of rice leaves obviously. Similarly, Shu et al. (2019) also found that K contents in leaves

of *Jatropha curcas* L. are gradually decreased with decreasing pH value of simulated acid rain. Our results supported the above results that spraying acid rain – reduced K uptake and accumulation in flowering Chinese cabbage.

Effect of simulated acid rain on soil nutrients. Since long-term acid rain causes soil acidification (Zheng et al. 2018), acid rain not only inhibited the growth of flowering Chinese cabbage (Figure 1) but also influenced soil chemical properties (Figure 4). As shown in Figure 4, spraying 7 times of pH 3.5 SAR decreased soil pH at a depth of 0, 4, and 8 cm by 0.21, 0.19, and 0.15 units in comparison to the control. Remarkably, no difference in soil pH was observed among treatments with pH 4.5 SAR, pH 5.5 SAR, and the control. Li et al. (2019) studied the impact of simulated acid rain on the soil of mixed

coniferous-broadleaved forest in the Three Gorges Reservoir Area of Jinyun Mountain and found that the treatment with SAR at pH 3.25 and 2.5 reduce soil pH by 5.18% and 9.07%, respectively, which was in accordance with our results (Figure 4A).

According to the acid rain data released by the Guangdong Meteorological Bureau of China in 2017, we set the amount of acid rain to 96.6 mL each time. Since the amount of acid rain mainly moistened soil layer from 0 cm to 10 cm, soil pH values of 12 cm and 16 cm soil depth were not different among different SAR treatments (Figure 4A). Wei et al. (2020) found that simulated acid rain treatments significantly reduce soil pH by 6.8, 7.0, and 5.1% in red soils, lateritic red soils, and latosols, respectively. Wu et al. (2016) conducted simulated acid rain treatments on southern subtropical forest soil of China twice

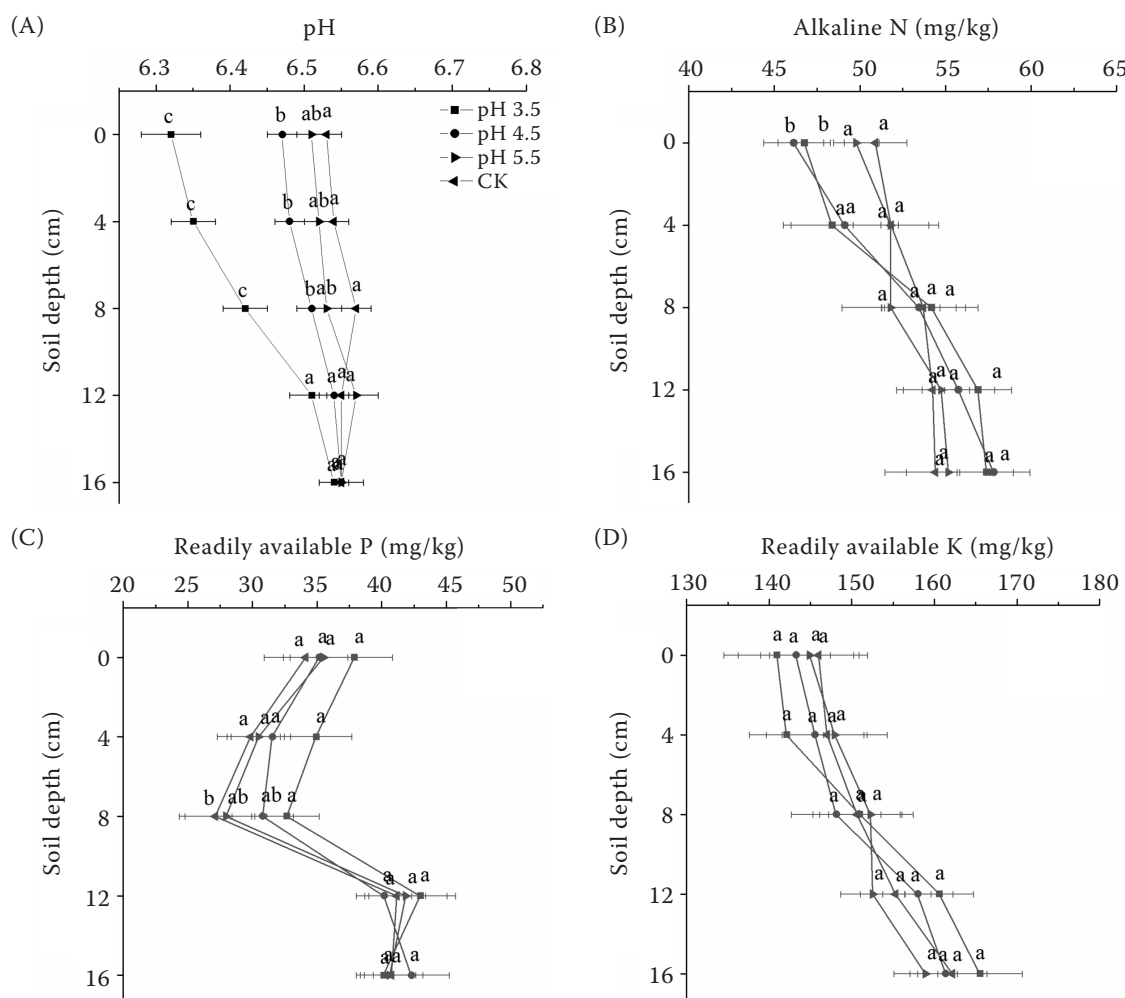


Figure 4. Variation in soil pH and available nutrients under simulated acid rain (SAR) stress at different soil depths. (A) pH; (B) alkaline nitrogen; (C) readily available phosphorus, and (D) readily available potassium. The horizontal line represented pH or nutrient content. Different letters of the same soil depth indicated significant differences at $P < 0.05$ according to Duncan's test; CK – control

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a month and found that 24 months of SAR treatment significantly decreases the pH value of soil in the broad-leaved forest and mixed forest. These results suggested that the acidification effect of acid rain on soil depends on the intensity, frequency and duration of acid rain and soil properties.

Soil available nutrients are the nutrient fractions in the soil that can be directly absorbed and utilised by plants, often referring to soil alkaline-hydrolytic N, readily available P and readily available K (Mishima et al. 2013). In this study, the changes of available nutrients at different depths in the rhizosphere of flowering Chinese cabbage were measured in response to different SAR treatment as shown in Figures 4B–D. It was observed that treatment with SAR at pH 3.5 reduced the contents of alkaline-hydrolytic N and readily available K obviously in the 0 cm to 8 cm soil layer in comparison to the control (Figures 4B, D). This was similar to the results of Cho et al. (2002). The lower the pH value of acid rain, the more severe the leaching of N and K in 0 cm to 10 cm soil occurred. Interestingly, the contents of alkaline-hydrolytic N and readily available K in 10 cm to 16 cm soil layer were slightly higher than those of the control. These results suggested that acid rain could increase N and K mobility, which promoted the movement of N and K from the upper soil to the lower soil.

On the contrary, the content of readily available P in 0 cm to 8 cm soil layer increased by 8.50% to 14.93% under SAR at pH 3.5 treatment (Figure 4C). Xu et al. (2015) found that acid rain with pH < 3.0 enhances the release of soil P, which contributes to the elevation of the content of soil readily available P (Turner et al. 2013). Among their reports, the contents of available phosphorus were increased by 49.18% and 12.71%, respectively, in response to pH

2.5 and pH 3.0 treatments. Our results supported the above view that acid rain at pH 3.5 treatment increased the content of soil readily available P and enhanced the transformation of soil P in surface soil layers (Figure 4, Table 1).

Soil phosphorus fractions. Inorganic phosphates in the soil can be classified into four main groups: calcium phosphate (Ca-P), aluminum phosphate (Al-P), iron phosphate (Fe-P), and reductant-soluble phosphate (Cho et al. 2002). The result of the P fraction analysis of the surface soil at a depth of 0 cm to 8 cm was performed, as shown in Table 1. Wu et al. (2016) found that a high concentration of protons is beneficial to the release of P from poorly soluble phosphates and facilitates soil organic carbon accumulation. This reason probably explains that treatment with pH 3.5 SAR elevated the content of readily available P in the surface soil layer (Figure 4C). All SAR treatments did not influence the content of total P in surface soil. No difference in total P was observed among different SAR treatments. Organic P did not vary greatly in response to different SAR treatments (Table 1). It was noteworthy that treatment with pH 3.5 SAR enhanced the content of readily available P in comparison to other SAR treatments. Fe-P, Al-P, and Ca-P with pH 3.5 SAR treatment were slightly reduced compared with those of other treatments. Olsen-P content did not change obviously in response to different SAR treatments. Turner et al. (2013) found that the leaching rate of soil P is directly related to the pH value of acid rain. The acidification of soil accelerates the solubilisation of iron- and aluminum-bound phosphates, which promote the release of soil P. Cho et al. (2002) found that acid rain can significantly change the P fraction of the soil and decrease the content of Fe-P by about 30%. In this study, slight decreases of

Table 1. Different phosphorus (P) components in rhizosphere soil of flowering Chinese cabbage under simulated acid rain treatment

Treatment	Total P	Organic P	Available P	Al-P	Fe-P	Ca-P	O-P
	(mg/kg)						
pH 3.5	447.6 ^a	175.9 ^a	50.2 ^a	38.8 ^a	87.3 ^a	49.8 ^a	58.7 ^a
pH 4.5	432.1 ^a	178.1 ^a	45.4 ^b	40.8 ^a	94.4 ^a	50.1 ^a	58.3 ^a
pH 5.5	469.0 ^a	177.4 ^a	42.3 ^b	42.1 ^a	97.9 ^a	51.4 ^a	59.5 ^a
CK	458.3 ^a	180.8 ^a	42.9 ^b	42.2 ^a	99.9 ^a	51.4 ^a	60.1 ^a

Values followed by a different lower-case letter in the same vertical column were statistically different ($P < 0.05$; Duncan's test). Total P – total phosphorus; organic P – organic phosphorus; available P – available phosphorus; Al-P – aluminum-bound phosphate; Fe-P – iron-bound phosphate; Ca-P – calcium-bound phosphate; O-P – occluded phosphorus; CK – control

soil Al-P and Fe-P contents might be associated with the intensity and treatment duration of SAR, and soil properties etc., and the underlying mechanisms are worthy of further study.

In conclusion, different from grain and economic crops and trees, there is little studies focus on the damage of acid rain to vegetables, especially cabbage; and those studies often paid attention to the secondary damage due to acid-activated heavy metals (Xiong and Wang 2005, de Freitas-Silva et al. 2016) or nutrient accumulation in plant individually (Fang et al. 2013). In this study, both direct damage to flowering Chinese cabbage in morphological and physiological aspects and indirect disturbance in soil nutrient availability. Besides, we provided dynamic results of these parameters with increasing exposure times, which were seldom mentioned in previous studies.

It can be summarised from the results of Figures 1 to 3 that physiological and morphological damages of cabbage and nutrient uptake hindering occurred with different extents as pH of rain lower than 5.5, but the differences among treatments were insignificant after being exposed to acid rain once or twice for most parameters. So the damages can be alleviated if proper rescue means, e.g., plastic film covering and foliar applying alginate oligosaccharides (Liu et al. 2013, Salachna et al. 2019) applied after the first acid rain exposure.

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