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Original article

The effect of curcumin on the physiological resistance and DNA methylation level of grape cultivar 'Shuijing' under the aluminum stress

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Summary

Al toxicity is the main limiting factor for crop production in acidic soil, so this study is aimed to improve understanding of the effects of curcumin on the aluminum (Al) tolerance of grapes, Al-tolerant cultivation and the epigenetic mechanism of grapes exposed to Al. *Vitis vinifera* × *V. labrusca* 'Shuijing' cuttings were cultivated in greenhouse, which were exposed to 20 mmol L^{-1} aluminum sulfate and then treated with curcumin in different concentrations. Then, indicators of physiological resistance and the DNA methylation level of the grape leaves were measured. The results demonstrated that Al stress led to a series of physiological and biochemical changes in grape leaves and significantly increased DNA methylation levels. Specifically, the chlorophyll, protein, phosphorus (P) and potassium (K) content decreased, activity of superoxide dismutase (SOD) and peroxidase (POD) also decreased, while the proline, malonaldehyde and Al content increased drastically, resulting in damage to grape plants. However, treatment by 100 and 200 µmol L^{-1} of curcumin led to significantly reduced DNA methylation levels and Al accumulation in grape leaves, reduced accumulation of malonaldehyde and proline, increased chlorophyll and protein content, enhanced SOD and POD activity, and improved intake of P and K. In summary, treatment by 100 and 200 µmol L^{-1} curcumin had a significant effect on the Al tolerance of grapes, indicating that curcumin treatment may be an effective solution to Al toxicity for grape cultivation in acidic soil.

Keywords
aluminum toxicity, antioxidant enzymes, curcumin, DNA methylation levels, grape, osmotic regulating substance

Significance of this study

What is already known on this subject?

- In acidic soil, Al toxicity is the main limiting factor for crop production. As a DNA methylation inhibitor, curcumin enhances plant resistance by reducing its DNA methylation level. Indeed, curcumin has been widely used for plant resistance to low temperatures, draughts and other abiotic stresses.

What are the new findings?

- Curcumin can significantly enhance the Al tolerance of grape plants under Al stress (20 mmol L^{-1}). Under Al stress, treatment by 100 and 200 µmol L^{-1} curcumin led to significantly reduced DNA methylation levels and Al content, and significantly increased chlorophyll content and SOD and POD activity, demonstrating that curcumin plays a key role in the Al tolerance of grapes.

What is the expected impact on horticulture?

- The results demonstrate that curcumin can effectively enhance the Al tolerance of grapes. Additionally, this study helps to clarify the physiological resistance and the epigenetic mechanisms of Al tolerance in grapes, thus facilitating grape cultivation in acidic soil with severe Al toxicity.

Introduction

Aluminum (Al) accounts for about 7.5–8.0% of the total mass of the earth's crust (Silambarasan et al., 2019). In neutral or alkaline soil, aluminum is not easily absorbed and has no toxic effect on plants (Bates et al., 1973). However, in acidic soil, a large amount of aluminum dissolves in the soil solution to form Al^{3+}. Acidic soil covers about 40% of the world's total land area and 70% of the world's potential arable land area. In China, red soil is mainly rich in aluminum, accounting for about 20% of the total land area (Guo et al., 2010). Aluminum toxicity mainly results in the inhibition of root growth, affecting the absorption of water and nutrients, and the accumulation of active oxygen (ROS). It has been demonstrated that under Al stress, the generation of oxygen free radicals is one of the main causes of oxidative tissue damage and an abnormal metabolism in an organism. The production of oxygen free radicals can destroy the structure of nucleic acids, chloroplasts and cell membranes, and consequently, reduce crop yield (Giannakoula et al., 2010; Yang et al., 2011). Due to the frequent occurrence of acid rain in China and the application of a large amount of acidic fertilizers, soil acidification has intensified and the dissolution of Al^{3+} has increased, resulting in an obvious increase in the amount of aluminum activity in the soil, which substantially restricts the growth of plants. Therefore, there is an urgent need to provide a solution for the toxicity of aluminum in acidic soil.

DNA methylation is an important form of epigenetic modification. It causes differences in the expression of plant-specific genes under adverse environments, suggesting that methylation plays an important regulatory role in breeding resistance. DNA methylation inhibitors, as an important means of regulating methylation imbalance, affect the flowering, growth and stress resistance of plants by changing

the DNA methylation level of the plant's genome (Miao et al., 2021). Recently, curcumin has attracted great attention in virtue of its powerful anti-inflammatory and antioxidant properties. Curcumin is an important component in turmeric, and combines irreversibly with DNA methyltransferase making it difficult for the genome to maintain a high state of methylation. Curcumin has the advantages of having a simple structure and being non-toxic, and is a newly discovered stable and safe methylation inhibitor (Liu et al., 2017). As a chain-breaking natural antioxidant (Ak and Gul, 2008) and fat-soluble polyphenol antioxidant (Banerjee et al., 2008), curcumin has a variety of biological functions, such as antioxidation and scavenging free radicals, and is superior to vitamin E at inhibiting oxidative stress (Zhao, 2020). More importantly, curcumin is a potential DNA methylation transferase (DNMTs) inhibitor and it can effectively control DNA methylation at a nanomolecular level (Lyko and Brown, 2005).

Vitis vinifera × *V. labrusca* 'Shuijing' hybrids (a grape cultivar) is rich in nutrition and has anti-cancer and anti-aging effects. The cultivar has high economic benefits because of its early maturation and high yield. However, if the grapes are cultivated in acidic soil, under Al stress, they produce stress reactions such as infiltration and oxidation, which inhibit their growth (Zhang et al., 2020). In the southern part of China, the soil is acidic red soil and aluminum toxicity has a great impact on the development of the grape industry. Aluminum toxicity has attracted significant attention from researchers. At the same time, with the continuous improvement of molecular technology and the increase in epigenetic research, DNA methylation which plays an important regulatory role in plant stress resistance, has increasing research significance. Nevertheless, studies of the resistance enhancement of curcumin focus on kiwi fruit (Hua, 2019), corn (Kimatu, 2010) and chrysanthemum (Zhao, 2015), while studies on grapes are rare. Consequently, we aimed to determine the effect of curcumin on grape cultivation in acidic soil. In this study, 'Shuijing' were used as the experimental material. The material was treated with up to 20 mmol L^{-1} of aluminum sulfate, and different concentrations of curcumin were tested for alleviating the damage caused by aluminum toxicity. This provided a theoretical basis to further analyze the regulation of physiological aluminum-tolerance and the epigenetic mechanism caused by curcumin, as it is of great significance to grape cultivation in acidic soil.

Materials and methods

Materials and processing

Vitis vinifera × *V. labrusca* 'Shuijing' robust branches were provided by Dongfeng Farm Administration Bureau of Mile City (long. 103°43'; lat. 24°41'), Yunnan Province. The branches were pruned in winter, the morphological upper end was cut into a flat mouth and the lower end into an oblique mouth, leaving 2–3 axillary buds in each branch segment. The morphological lower end of the branches was then inserted into a seedling bag containing substrate. When the seedling cuttings grew to 40–50 cm, the seedlings that had strong and consistent growth and were free of disease and insect pests were selected and planted into a nutrient bowl with substrate (peat:vermiculite:perlite = 3:1:1). One plant was planted per bowl (24 × 27 cm), and 500 mL of Hoagland's nutrient solution (pH = 4.8) (Li et al., 2023) was added every three days.

In this study, the plants were divided into six different treatment groups with aluminum sulphate ($Al_2(SO_4)_3$) and curcumin (CUR) treatment combinations, including: the control group (receiving no treatment with $Al_2(SO_4)_3$ or CUR), 20 mmol L^{-1} $Al_2(SO_4)_3$, 20 mmol L^{-1} $Al_2(SO_4)_3$ and 100 µmol L^{-1} curcumin (Al+CUR100), 20 mmol L^{-1} $Al_2(SO_4)_3$ and 200 µmol L^{-1} curcumin (Al+CUR200), 20 mmol L^{-1} $Al_2(SO_4)_3$ and 400 µmol L^{-1} curcumin (Al+CUR400), and 20 mmol L^{-1} $Al_2(SO_4)_3$ and 800 µmol L^{-1} curcumin (Al+CUR800) (Supplemental Table S1). For the treatments that received 20 mmol L^{-1} $Al_2(SO_4)_3$, it was added to Hoagland's nutrient solution (pH = 4.8) and replaced once a week. Each plant that received curcumin was sprayed with curcumin at different molar concentrations, evenly on the front and back of the plant's leaves and stems, once every three days. Each treatment group consisted of four plants, and the treatment period was 35 days. Samples were taken on day 0, 7, 14, 21, 28 and 35, respectively. The mature, disease-free leaves in the middle of the grape plant were randomly selected for sampling, and the leaves were frozen quickly in liquid nitrogen and stored at -80°C for later use.

Measurement of pigments and malonaldehyde content in the leaves

On day 35, healthy leaves were collected from the middle of the plant for measurements of chlorophyll, carotenoid and malonaldehyde content and the fresh weight of the grape leaves. Chlorophyll and carotenoid content were measured by extraction using 95% ethanol. Fresh plant leaves were added to 95% ethanol, soaked until the tissue turned white, and then the optical density (OD) values were measured at 665, 649 and 470 nm wavelengths, then the calculations were done according to the protocol formula (Boddi et al., 2010). The malonaldehyde content was measured by two-component spectrophotometry. We took 2.0 mL of supernatant, added 2.0 mL of 0.5% thiobarbituric acid (TBA) solution, put it into a boiling water bath for 20 min and measured the OD values at 532, 600 and 450 nm wavelengths (Dhindsa et al., 1981). The fresh weight of the leaves was measured by the punching method. Dust was removed from the leaf surface, large leaf veins were avoided during sampling, five green leaves were punched with a 1-cm diameter leaf disc, and then they were weighed immediately.

Measurement of proline and protein content

Samples were collected on day 0, 7, 14, 21, 28 and 35. Mature and disease-free leaves in the middle of the grape plant were randomly selected to determine proline and protein content. The proline content was measured by absorbing 2 mL of supernatant, adding 2 mL of glacial acetic acid and 3 mL of color developing solution, boiling it in a water bath for 40 min, extracting the red substance with toluene, and conducting colorimetry at a wavelength of 520 nm (Boddi et al., 2010). The protein content was measured using the Coomassie brilliant blue method, conducting colorimetry at 595 mm, and recording the absorbance value (Bates et al., 1973).

The measurement of antioxidant enzyme activity

The superoxide dismutase (SOD) activity was measured using a 20 min color reaction under fluorescence, absorbance was measured at 560 nm (Beauchamp and Fridovich, 1971). The peroxidase (POD) activity was measured by adding 3 mL of the reaction mixture and 0.1 mL of the enzyme solution, shaking it well, recording the time immediately, and measuring at 470 nm (Ultraviolet Spectrophotometer

C-7100) every 30 s. We used a change of 0.01 in A470 per minute as a POD activity unit (Lagrimini, 1991).

Measurement of nitrogen, phosphorus, potassium and aluminum content

After drying, samples were crushed and sieved through a standard 100-mesh sieve and the powder was digested with sulfuric acid (H_2O_2) to determine nitrogen (N), phosphorus (P) and potassium (K) content (Osaki, 1995). Specifically, N content was measured using Nessler's reagent color method. We put 1 mL of the test solution into a 25 mL volumetric flask, and successively added 1 mL of 25% potassium sodium tartrate, 15 mL of ammonia-free distilled water and 1 mL of Nessler's reagent. The mixture was shaken well, and after five minutes colorimetry was performed at the wavelength of 480 nm. Phosphorus content was measured using the molybdenum blue method. We took 0.5 mL of the test solution, 0.5 mL of diluted water, and 4 mL of mixed color developing solution and put it into a water bath for 30 min, and colorimetry was conducted at a wavelength of 680 nm. Potassium content was measured using the sodium tetraphenylborate method and colorimetry was conducted at a wavelength of 630 nm. Aluminum content was measured using Chromazurol S spectrophotometry. Samples were burned to white gray, hydrochloric acid was added, the mixture was shaken well, 1.0 mL of the sample solution and 4 mL of distilled water were adjusted to 10 mL using pure water, the mixture was mixed well, and colorimetry was conducted at a wavelength of 620 nm (Yu, 2012).

Sequencing and analysis of the DNA methylation level

Using the QIAamp Fast DNA Tissue Kit (Qiagen, Düsseldorf, Germany), the methods were conducted according to the procedures provided by the manufacturer. The total DNA of control, Al 20 mmol L^{-1}, and Al+CUR100 samples was extracted respectively, and then the qualified samples

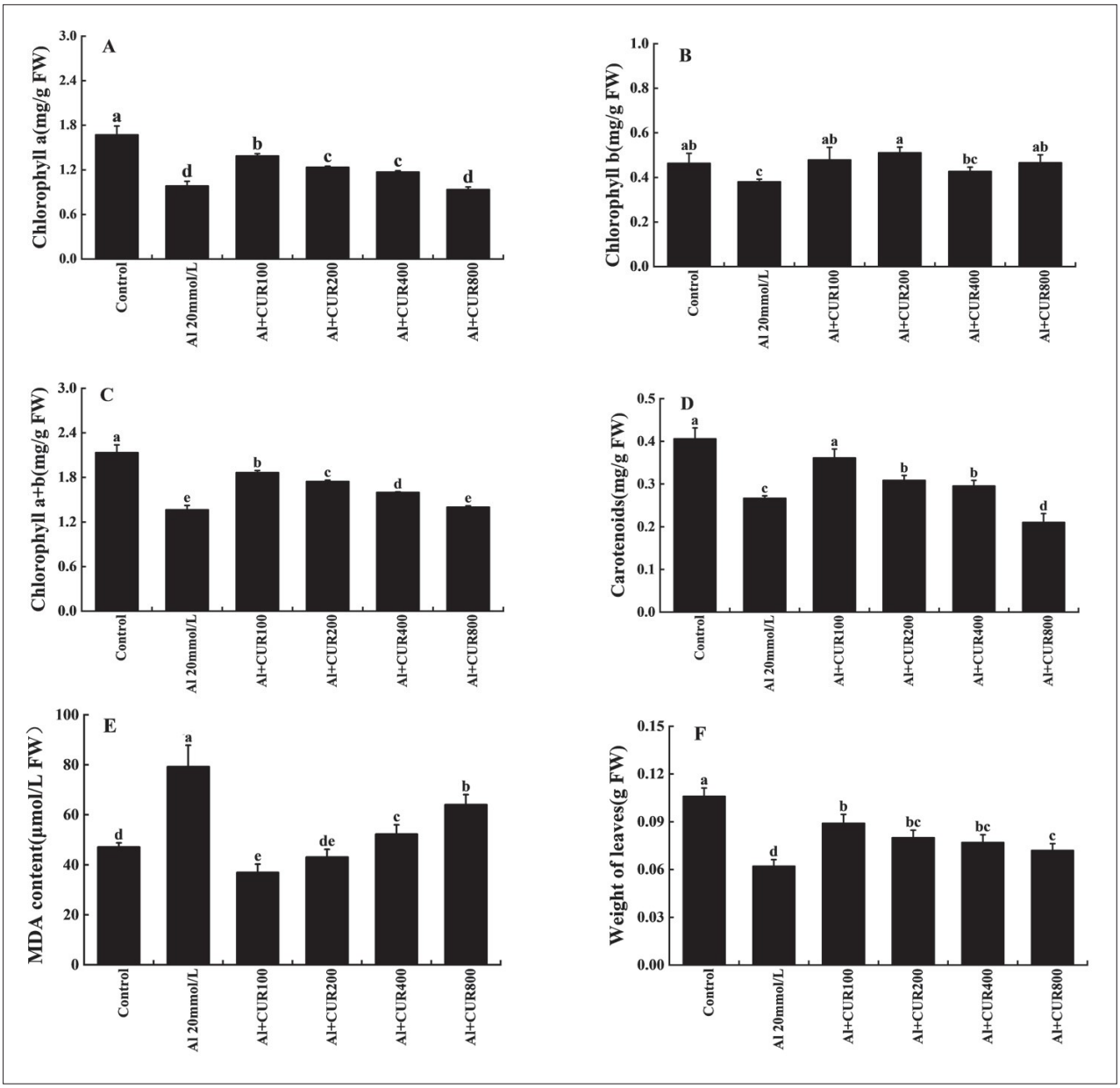


Figure 1. The effect of curcumin on chlorophyll *a* (A), chlorophyll *b* (B), chlorophyll *a*+*b* (C), carotenoid (D) and malonaldehyde (E) content and the fresh weight of grape leaves under Al stress (F). The histogram shows the mean (± standard deviation) of three repeated tests of the results of Tukey's HSD test. Different lowercase letters indicate that the difference is statistically significant ($p < 0.05$).

were segmented by ultrasound for bisulfite conversion and second-generation sequencing. The Accel-NGS methylseq DNA library kit was used to screen and connect the transformed DNA fragments to construct the library. The HiSeq4000 sequencing platform 150PE was used for sequencing. During the construction of the DNA library, bisulfite was used as the control group.

Data processing

The data of content of pigments, malonaldehyde, proline, protein, nitrogen, phosphorus, potassium and aluminum, the activity of antioxidant enzyme, and the level of chromosome DNA methylation were used analysis by SPSS19.0 software. The Duncan's new complex difference method was used to test for the data significance ($P < 0.05$), and the Origin 2019 software to plot the results.

Results and analysis

The effect of curcumin on chlorophyll and malonaldehyde content and the fresh weight of grape leaves

Under Al stress, the chlorophyll content of grape leaves decreased drastically compared with the control group. Curcumin treatments were found to relieve such a decrease. Specifically, chlorophyll *a*, chlorophyll *a+b* and carotenoid content were inversely proportional to the CUR concentration. Indeed, chlorophyll *a*, chlorophyll *a+b* and carotenoid content of the CUR100, CUR200 and CUR400 treatment groups were significantly higher than in the Al stress treatment group, especially the CUR100 treatment group (Figure 1A, C, D). Chlorophyll *b* content in groups other than CUR400 was significantly higher than in the Al 20 mmol L^{-1} (Figure 1B). Under Al stress, the malonaldehyde content of the grape leaves increased, compared to the control group; after CUR treatment, the malonaldehyde content of the grape leaves dropped drastically. In particular, the malonaldehyde content of the CUR100 group was significantly lower than in other groups (Figure 1E). Under Al stress, the fresh weight of the grape leaves decreased significantly compared to the control group; after CUR treatment, the fresh weight of the grape leaves increased significantly, especially in the CUR100 treatment group (Figure 1F). In summary, treatments by 100 and 200 µmol L^{-1} of CUR significantly reduced malonaldehyde content, and enhanced chlorophyll content and the fresh weight of the grape leaves, thus improving the Al tolerance of the plants and relieving the grape seedings of Al toxicity.

The effect of curcumin treatments on enzyme activity in grape leaves

To cope with adverse stress, plants have evolved an antioxidant enzyme system. As the key enzymes for scavenging active oxygen in plants, SOD and POD maintain the dynamic balance of active oxygen and avoid damage to the biofilm from free radical accumulation.

On day 0, the SOD and POD activity of different treatment groups was negligibly different (Figure 2A, B). In terms of SOD activity, on day 7–35, the SOD activity of the control group was significantly higher than in the other groups, except for that of the CUR100 treatment group on day 21. On day 7–14, the SOD activity in the CUR100, CUR200 and CUR400 treatment groups was significantly higher than in the Al 20 mmol L^{-1}. On day 21–35, the SOD activity of all treatment groups was significantly higher than in the Al 20 mmol L^{-1}, while the SOD activity of the CUR100 treatment group was higher than in the CUR200, CUR400 and CUR800 treatment groups (Figure 2A). Looking at both POD and SOD activity, on day 7, the POD activity of the CUR100 treatment group was higher than in the other groups, whereas on day 14–21, SOD and POD activity of the CUR100 treatment group

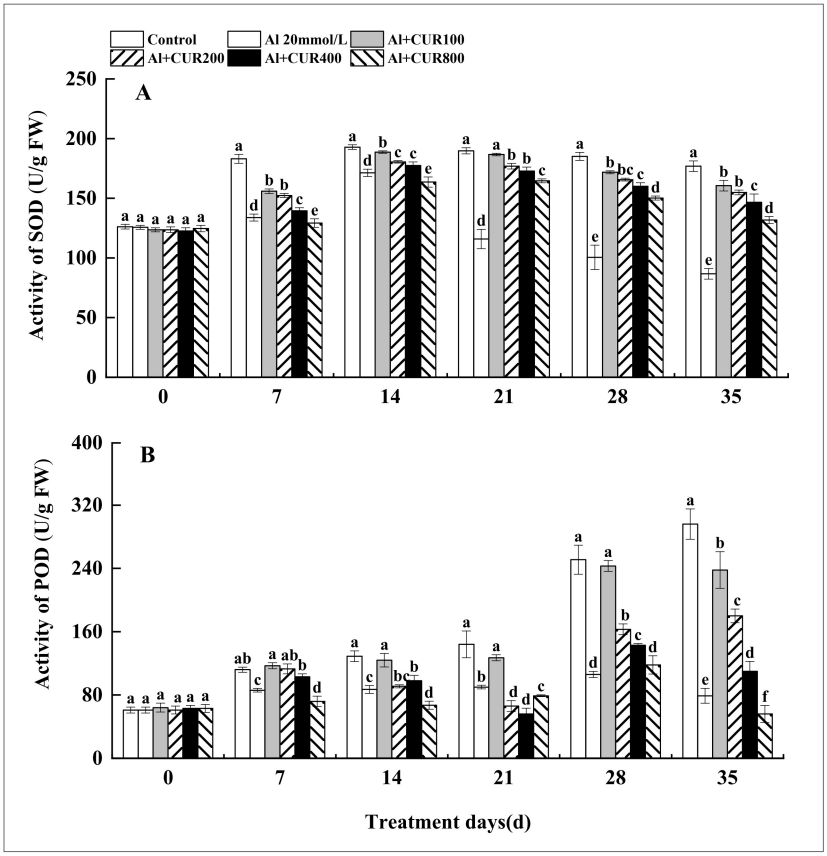


Figure 2. The effect of curcumin on superoxide dismutase (SOD) (A) and peroxidase (POD) (B) activity in grape leaves under Al stress. The histogram shows the mean (± standard deviation) of three repeated tests of the results of Tukey's HSD test. Different lowercase letters indicate that the difference is statistically significant ($p < 0.05$).

was lower than in the control group, but significantly higher than in other groups. Then, on day 28–35, SOD and POD activity of the CUR100, 200, 400 and 800 treatment groups were lower than in the control group, but significantly higher than in the Al stress group (Figure 2B). In summary, Al stress led to reduced SOD and POD activity in grape leaves, while CUR treatment relieved such degradation, especially in the CUR100 treatment group, thus playing a key role in the enhancement of Al tolerance in grapes.

The effects of curcumin on nitrogen, phosphorus and potassium content

On day 0, N, P and K content in all groups were negligibly different (Figure 3A–C). In terms of N content, on day 7–14, the Al stress group had the highest Al 20 mmol L^{-1}, followed by the CUR100 treatment group. On day 21, the N content of the CUR100 treatment group was significantly higher than in the other groups, while the Al 20 mmol L^{-1} group had the lowest N content. On day 28, the N content of the CUR100 treatment group and the control group was significantly higher than in the other groups. Then, on day 35, the CUR100 treatment group were significantly higher than in the groups other than the control group, while the Al 20 mmol L^{-1} were significantly lower than in the other groups (Figure 3A). In terms of P, on day 7, the P content of the Al stress group was significantly lower than in the control group, and the CUR100 and CUR200 treatment groups, while the P content of the CUR100 and CUR200 treatment groups was significantly higher than in the control group. On day 14–35, the P content of the Al stress group was significantly lower than in the other groups, while the P content of the CUR100 treatment group was significantly higher than in the CUR200, CUR400 and CUR800 treatment groups (Figure 3B). In terms of K, on day 7, K content of the control group and the CUR100 treatment group was significantly higher than in the Al 20 mmol L^{-1}. On day 14, the K content of groups other than the CUR200 treatment group was significantly higher than in the Al 20 mmol L^{-1}. On day 21–28, the K content of the Al 20 mmol L^{-1} was significantly lower than in the other groups. Then, on day 35, the K content of the Al 20 mmol L^{-1} was significantly lower than in the groups other than the CUR800 treatment group, while the K content of the CUR100 treatment group was significantly higher than in the other groups. In summary, Al stress inhibited accumulation of N, P and K in the grape leaves, while CUR treatments relieved this inhibition. Specifically, the CUR100 and CUR200 treatments were effective and had a positive effect on the Al tolerance of grapes.

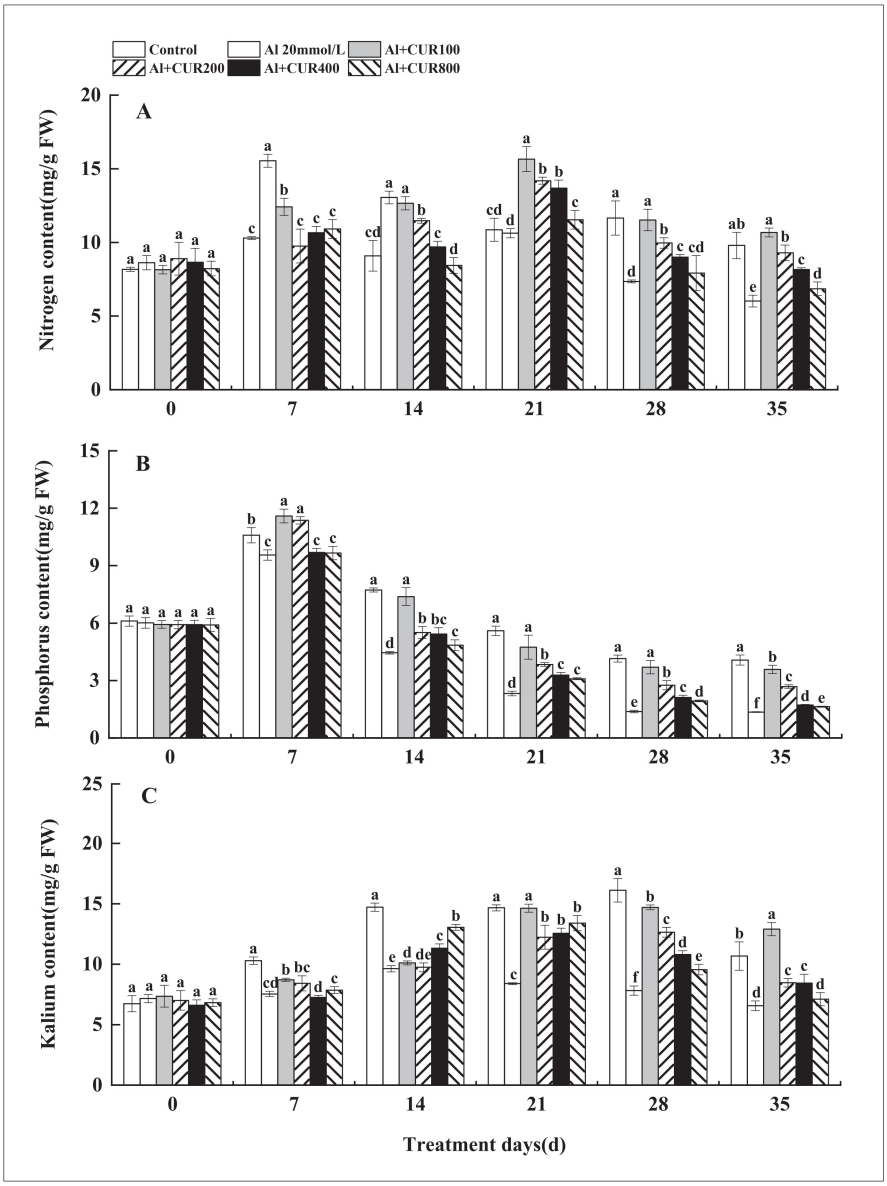


Figure 3. The effects of curcumin on nitrogen (A), phosphorus (B) and potassium (C) content in grape leaves under aluminum stress. The histogram shows the mean (± standard deviation) of three repeated tests of the results of Tukey's HSD test. Different lowercase letters indicate that the difference is statistically significant ($p < 0.05$).

The effect of curcumin on aluminum, proline and protein content

On day 0, the Al, proline and protein content in all groups was negligibly different (Figure 4A–C). In terms of Al content, on day 7, the Al content of the CUR800 treatment and Al 20 mmol L^{-1} was significantly higher than in the other groups, on day 14, the Al content of the CUR100 treatment group was significantly lower than in the other groups. On day 21, the Al content of the CUR100 treatment group was lower than in the control group and significantly lower than in the other groups. On day 28, the Al content of the control group was significantly lower than in the other groups, while the Al content of these groups followed the sequence of Al > CUR800 > CUR400 > CUR200 > CUR100 and inter-group differences were significant. On day 35, the Al content of the CUR800 treatment group was significantly higher than in the other groups (Figure 4A). In terms of proline content, on day 7–35, the proline content of the Al stress group increased drastically and proline content in Al 20 mmol L^{-1} was significantly higher than in the control group, while proline content in the CUR100 and CUR200 treatment groups was significantly lower than in the Al 20 mmol L^{-1}. On day 28, proline content of the CUR400 and CUR800 treatment groups was higher than in the Al 20 mmol L^{-1} (Figure 4B). Looking at protein content, on day 7–35, the protein content of the control group was higher than in the other groups. On day 7–28, the protein content of the Al 20 mmol L^{-1} was significantly lower than in the other groups. Then, on day 35, the protein content of CUR100, CUR200 and CUR400 treatment groups was higher than in the Al 20 mmol L^{-1}, while the protein content of the CUR800 treatment group was significantly lower than in the Al 20 mmol L^{-1} (Figure 4C). In summary, the CUR100, CUR200, CUR400, and especially the CUR100 treatment groups relieved the effects of Al toxicity by significantly reducing the accumulation of Al and proline, and accelerating protein synthesis. Therefore, curcumin treatment was shown to improve Al resistance in grape seedlings.

The effects of curcumin on DNA methylation levels

Under Al stress, the DNA methylation levels of Chromosome 1, Chromosome 2, Chromosome 3, Chromosome 4, and Chromosome 5 (Chr1, Chr2, Chr3, Chr4, and Chr5) were enhanced by 24.11%, 11.67%, 11.13%, 21.93%, and 19.67%, respectively, compared to the control group. Meanwhile, the DNA methylation levels of Chr1, Chr2, Chr3, Chr4 and Chr5 in the CUR100 treatment group decreased by 50.67%, 17.81%, 22.32%, 13.68% and 22.84%, respectively, compared to the Al 20 mmol L^{-1}. Also, DNA methylation levels of Chr1, Chr2,

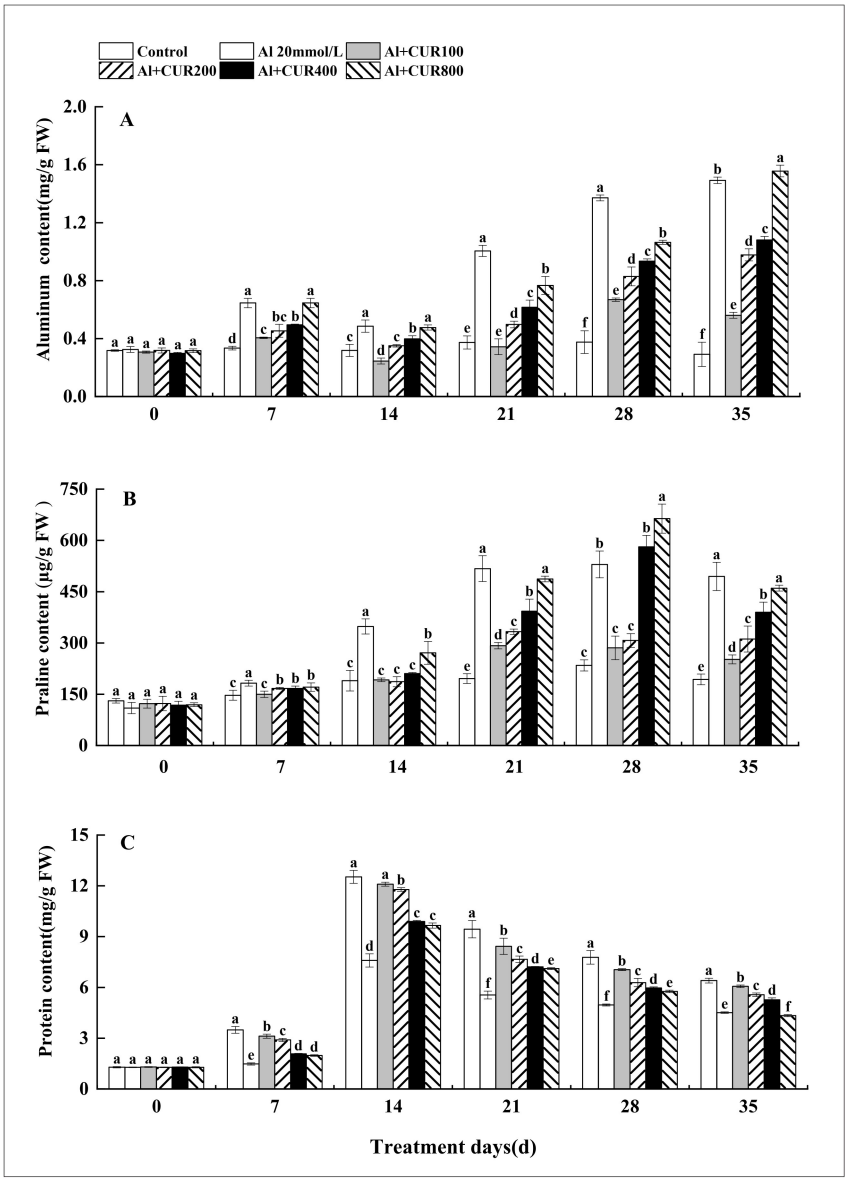


Figure 4. The effects of curcumin on aluminum (A), proline (B) and protein (C) content in grape leaves under aluminum stress. The histogram shows the mean (± standard deviation) of three repeated tests of the results of Tukey's HSD test. Different lowercase letters indicate that the difference is statistically significant ($p < 0.05$).

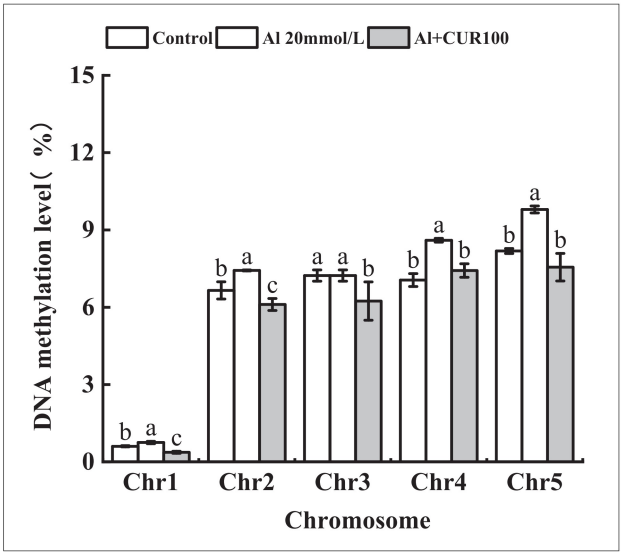


FIGURE 5. The effects of curcumin on DNA methylation levels in grape leaves under aluminum stress. The histogram shows the mean (± standard deviation) of three repeated tests of the results of Tukey's HSD test. Different lowercase letters indicate that the difference is statistically significant ($p < 0.05$).

Chr3 and Chr5 in the CUR100 treatment group were 38.77%, 8.22%, 13.68% and 7.66% lower than in the control group (Figure 5). Therefore, DNA methylation in grape leaves was found to have a close relationship with adaption to Al stress. Treatment with CUR100 enhanced Al tolerance in grapes by reducing the DNA methylation level of grape leaves under Al stress.

Discussion

The effect of curcumin on chlorophyll and malonaldehyde content and the fresh weight of grape leaves

To survive in environments that are high in toxic aluminum such as acidic soil, plants have developed mechanisms of external exclusion and internal tolerance. In aluminum-accumulating plants such as buckwheat (Ma et al., 1997) and hydrangea (Ma et al., 2001), the internal tolerance mechanism is the chelation of oxalate and citrate. In the leaves, aluminum is isolated into vacuoles and appears in the form of aluminum oxalate in the ratio of 1:3. Photosynthesis is considered to be one of the processes that is sensitive to Al stress, which eventually leads to the disintegration of cell membranes and the degradation of cell components (Shen and Ma, 2001). Chlorophyll plays a role in accepting and converting energy during photosynthesis, and its content is closely related to photosynthetic levels. Malonaldehyde is one of the common products of a lipid peroxidation reaction, and its content reflects the level of free radicals. Plant leaves are the organs that are most sensitive to environmental change and the main place for physiological and metabolic activities. In this study, Al stress led to significantly decreased chlorophyll content and fresh weight of grape leaves, and significantly increased malonaldehyde content. The absorption of malonaldehyde due to Al stress indirectly affected chlorophyll synthesis and leaf fresh weight. Treatment with curcumin promoted chlorophyll synthesis, alleviated cellular lipid peroxidation, reduced the malonaldehyde concentration and enhanced the fresh weight of the grape leaves, with 100 µmol L^{-1} of curcumin having the most significant effect.

The effect of curcumin on enzyme activity

A micromolar concentration of aluminum can damage plant root growth and inhibit cell division in the long term. Aluminum stress induces the accumulation of active oxygen (Richards et al., 1998). The enzymes, SOD and POD are important components of an organism's antioxidant system, and an increase in their activity can gives plants resistance to Al toxicity. Superoxide dismutase catalyzes the disproportionation of superoxide anion free radicals into H_2O_2 and oxygen (O_2), and POD decomposes H_2O_2 into water (H_2O) and O_2 to protect cells from oxidative attack. In this study, 100 µmol L^{-1} of curcumin significantly alleviated the reduction of SOD and POD activity induced by Al stress. This may be because curcumin can inhibit the binding of excessive aluminum to some biological macromolecules in cells, balance the increase of oxygen free radicals in cells and the free radical decomposition ability of cells themselves, and protect cell membranes from adverse effects.

The effect of curcumin on nitrogen, phosphorus and potassium content

The normal absorption and transportation of mineral elements is very important for plant growth and development. The combination of aluminum and phospholipids can inhibit the transport of cations by membrane, resulting in the lack of mineral elements in plants (Yu, 2012). In this study, Al accumulation under Al stress weakened the ability of the mineral elements P and K to be transported upward from the roots, weakened the absorption of P and K, and reduced its accumulation in the leaves. 100 and 200 µmol L^{-1} of curcumin was found to alleviate Al accumulation in grape leaves with a significant and stable effect, so that grapes were able to resist Al toxicity and maintain the transport of mineral elements, thereby enhancing the resistance of grape plants to Al.

The effect of curcumin on aluminum, proline and protein content

Aluminum has a low toxicity and is a non-essential element in neutral or alkaline soil, but it has direct phytotoxicity and has indirect physiological barrier effects on plants in acidic soil, which greatly weakens plants' resistance to Al. In this study, the proline content of grapes increased drastically, while the protein content decreased gradually under treatment with 20 mmol L^{-1} $Al_2(SO_4)_3$. However, when treated with 100, 200 and 400 µmol L^{-1} curcumin it led to decreased proline content, and the protein content increased as the proline content decreased. As it is initiated by protein degradation, proline accumulation in plants cannot play a protective role, but it is harmful to the crop itself (Devi et al., 2003).

The effect of curcumin on DNA methylation

Epigenetic changes are induced by stress in plants, allowing them to adapt to stress, leading to selection and evolution (Xing, 2014). DNA methylation plays an important role in regulating plant growth and development, and in the response of the plant to various stresses. However, high methylation has a low level of gene expression, and weakens plant resistance to adverse stress (Fan et al., 2019). In this study, the DNA methylation level increased significantly under Al stress, while curcumin could inhibit the increase to a varying degree. Aluminum stress leads to the increase of active oxygen concentration in grape leaves and accelerates the production of methyl-free radicals, thereby promoting the synthesis of 5-methylcytosine in DNA and increasing the DNA methylation level. However, curcumin irreversibly

binds to DNA methylation transferase, so the DNA methylation level decreases to a certain extent when grapes are treated with curcumin.

Conclusions

With the increase of Al stress over time, the Al tolerance of grapes decreased gradually, and various physiological indicators changed accordingly. Nevertheless, curcumin reduced Al toxicity by reducing the DNA methylation level of the grape leaves to alleviate the degree of damage to the plant. The potential mechanism may be that curcumin maintains the integrity of the genome and controls the expression of genes in cells, thereby enhancing the antioxidant capacity of grape plants, protecting the cell membranes from damage by oxygen-free radicals, reducing the accumulation of lipid peroxidation products, enhancing the accumulation of photosynthetic pigment and mineral elements in grape leaves, and maintaining physiological and metabolic activities of the plant, so as to improve the resistance and adaptability of grape plants to Al stress. Our results demonstrate that curcumin can effectively enhance the Al tolerance of grapes, therefore, spraying appropriate concentration of curcumin on grapes will effectively increase the tolerance of grapes to aluminum stress. Additionally, this study helps to clarify the physiological resistance and the epigenetic mechanisms of Al tolerance in grapes, thus facilitating grape cultivation in acidic soil with severe Al toxicity.

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Supplemental Information

Supplemental Information – Table S1. The content of aluminum sulphate ($Al_2(SO_4)_3$) and curcumin (CUR) for each treatment group.

Group name	The content of $Al_2(SO_4)_3$ (mmol L^{-1})	The content of CUR (μmol L^{-1})
Control	0	0
Al	20	0
Al+CUR100	20	100
Al+CUR200	20	200
Al+CUR400	20	400
Al+CUR800	20	800