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Physiological effects induced by aluminium and fluoride stress in tall fescue (*Festuca arundinacea* Schreb)

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ABSTRACT

Aluminium (Al) and fluoride (F) are phytotoxic elements that can inhibit plant growth and development. Al^{3+} and F^- can react with each other to form complexes in the soil which will induce alteration of toxicity of single element. However, the mechanisms of plant response to aluminium fluoride induced toxicity are not very clear. In the present study, tall fescue (*Festuca arundinacea* Schreb) cultivar 'Houndog 5′ was treated by 0, 0.4, 4, 20 $mg \cdot L^{-1}$ $Al_2(SO_4)_3$ and 0, 0.5, 5 $mg \cdot L^{-1}$ NaF, respectively. After 25 days of treatment, leaf samples were collected for physiological evaluation. The results showed that several forms of Al-OH and Al-F complexes such as $Al(OH)_2^{+}$, $AlOH^{2+}$, $Al(OH)_3$, $Al(OH)^{4-}$, $Al_2(OH)_2^{4+}$, $Al_3(OH)_4^{5+}$, AlF^{2+}, AlF_2^{+}, AlF_3 and AlF^{4-} were formed in Al^{3+} and F^- combined solution. The nutrient uptake including Al, P and K were improved by Al^{3+} and F^-. Under Al^{3+} stress, the MDA (malondialdehyde) content and EL (electrolyte leakage) dramatically increased after high concentration of F^- treatment, while relative low concentration of F induced decrease of MDA content and EL. On the contrary, chlorophyll content decreased significantly after high concentration of F treatment. The photosynthesis efficiency parameters, including φ_{P0} (Fv/Fm), δ_{R0} and PI_{ABS}, decreased remarkably after high concentration of Al and F treatment. However, L-band incresed after high concentration of Al^{3+} and F^- treatment. The results of correlation analysis showed that MDA content and EL negatively correlated with other indexes, and Al-F complex significantly correlated with MDA, Pro and EL but negatively correlated with Chl and φ_{P0}. These results suggested that low concentration of F could alleviate the damage induced by Al stress in tall fescue, but high concentration of Al and F combined solution had negative effects on the growth and development of tall fescue.

1. Introduction

Aluminium (Al) is a major element of soil, and it turns into toxic forms when expose to acidic condition. Al^{3+} ions are released from clay minerals in acidic soils, which in turn inhibit root growth and reduce crop yields. It is reported that approximately 40–50% potentially arable lands are acidic in the world (Von Uexküll and Mutert, 1995). Root is very sensitive to Al^{3+} toxicity and root meristem is the primary site of Al toxicity (Ryan et al., 1993). Root growth in wheat (*Triticum aestivum*) is inhibited within one hour after Al^{3+} treatment (Ownby and Popham, 1989). Negative effects induced by Al^{3+} on root will further decrease grain yields and disturb nutrition balance in plant. To tolerate Al^{3+} toxicity, plant has evolved different tolerance and detoxify mechanisms. In general, there are two types of Al^{3+} defense in plant, which are Al^{3+} excluders and Al^{3+} accumulators (Watanabe and Osaki, 2002). The Al^{3+} exclusion process takes place mainly via organic compounds exudation, such as organic acids including citrate, malate and oxalate (Kochian et al., 2004), and phenolic compounds (Kidd et al., 2001). The Al^{3+} accumulators which are Al^{3+} resistant species, for instance rice (*Oryza sativa*), buckwheat (*Fagopyrum esculentum*) and malabar melastome (*Melastoma malabathricum*) (Kochian et al., 2015; Osaki et al., 2003), can detoxify or translocate Al^{3+} in plant. Previous studies reported that the aluminium toxicity was attenuated by fluoride in wheat (*Triticum aestivum*) (MacLean et al., 1992). While, high concentration of fluoride (F) is toxic to plant (Barbier et al., 2010; He et al., 2021). Aluminium has the highest binding affinity with F among metal ions (Peng et al., 2021). It is

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reported that Al^{3+} is involved in alleviating F toxicity by forming Al-F complexes in tea (*Camellia sinensis*) (Yang et al., 2016). However, according to Kinraide's report, the Al-F complexes such as AlF^{2+} and AlF_2^+ are also toxic to plant (Kinraide, 1997). Aluminium ion can form some stable complexes with fluorinion which suggest a strong correlation between these two elements. Considering that fluorine is an important halogen in the environment, and the effects of Al^{3+} and F^- on plant is not very clear, therefore, it is necessary to investigate the effects of Al^{3+} and F^- on certain plant species.

When plants are exposed to adverse conditions, physiological changes occur in plant. The cell membrane is very sensitive to adverse conditions. As the indicators of cell membrane damage, malondialdehyde (MDA) and electrolyte leakage (EL) increase remarkably in plants under environmental stresses (Fan et al., 2015). For instance, the EL in bentgrass (*Agrostis stolonifera*) increased significantly after drought stress treatment (Ma et al., 2018). Photosynthesis and pigment content negatively changed in plants. The chlorophyll content and photosynthesis process in bermudagrass (*Cynodon dactylon*) were inhibited by salt stress (Fan et al., 2019). Besides, it is also reported that Al concentration has a negative relationship with many mineral concentrations in plants (Watanabe and Osaki, 2002). So, the ion homeostasis will change in plant when it is exposed to high Al concentration.

Tall fescue (*Festuca arundinacea* Schreb) is an important cool-season turfgrass which is grown in the temperate zone of the world. In the previous study, it showed phytoremediation potential of toxic elements after treated with cadmium (Huang et al., 2017). However, the effects of Aluminium fractions and its species on tall fescue are largely unknown. Therefore, the aim of this study is to illustrate the Al^{3+} and F^- response in tall fescue which will be contributed to extend application potential of tall fescue in diverse ecological restoration.

2. Materials and methods

2.1. Plant materials and growth conditions

Houndog 5, a popular cultivar of tall fescue (*Festuca arundinacea* Schreb) was used in this experiment. Before germination, the seeds were sowed in cubic plastic pots (10 cm side) with filled matrix (pearlite: vermiculite = 1:1). The 1 month old seedlings were transferred to Erlenmeyer flasks which were filled with half-strength Hoagland's nutrient solution for hydroponics. The temperature was 24/20 °C (day/night) for 16 h photoperiod. The light intensity was 300 μmol photons $m^{-2}\cdot s^{-1}$.

2.2. Al and F treatment

For solution preparation, 12 different Al-F mixed solutions were prepared. Briefly, $Al_2(SO_4)_3$ and NaF were dissolved into deionized water. To guarantee the uniformity of the solution, the Al^{3+} contents in the solution were adjust to 0, 0.4, 4 and 20 $mg\cdot L^{-1}$ (recorded as Al0, Al0.4, Al4 and Al20, respectively), F^- contents in the solution were adjust to 0, 0.5 and 5 $mg\cdot L^{-1}$ (recorded as F0, F0.5 and F5, respectively), in the Al-F mixed solution. For plant treatment, after growth in the prepared solution for one week, the leaves of the seedlings were submerged in different concentrations of $Al_2(SO_4)_3$ and NaF solutions for 2 h every day. The treatment lasted for 25 d. Five repeats were set in each treatment group. Leaf samples were collected after 25 days of treatment.

2.3. Detection of biomass

Fresh weight was measured by a balance at the end of treatment. The reading was recorded as biomass.

2.4. Detection of MDA content

MDA content reflects lipid peroxidation of cells. To detect the MDA content, extract solution is required (Gao, 2006). In brief, 0.1 g fresh leaves of tall fescue were ground into fine powder in liquid nitrogen. The powder was homogenized in sodium phosphate buffer (50 mM, pH 7.8) which was pre-treated in ice. Then the homogenate was centrifuged at 12, 000 g for 15 min at 4 °C. After that, 1 mL of the extract solution was mixed with 2 mL of reaction solution which contained 10% (v/v) trichloroacetic acid and 0.3% (v/v) thiobarbituric acid. The mixed solution was incubated in water bath at 95 °C for 30 min. After cooled to room temperature, the mixed solution was centrifuged at 3500 g for 10 min. The absorbance of the supernatant was detected at 450, 532 and 600 nm with a spectrophotometer. The MDA concent was calculated the formula: MDA content ($mol\cdot L^{-1}$) = 6.45 × (A532-A600) – 0.56 × A450 × V/W. V was volume of the supernatant, W was fresh weight of the sample.

2.5. Detection of relative EL

A puncher (5 mm diameter) was used to collect 0.1 g leaf samples in each treatment. The collected samples were immersed into 15 mL deionized water which was contained in 50 mL centrifuge tubes. After shaking for 24 h at room temperature, conductivity of the solution was measured and it was recorded as EL_a. Then the solution were autoclaved at 121 °C for 20 min. The conductivity was measured and recorded as EL_b. The relative electrolyte leakage (EL) was calculated as: $EL = EL_a/EL_b \times 100\%$.

2.6. Detection of ion content

The ion content detection was according to Zhu et al. (2020). In brief, the collected leaf tissues were dried at 105 °C for 30 min, and 70 °C for 2–3 days until the mass was constant. Then 0.1 g of the dried tissues were digested in 5 mL HNO_3 (assay 65%–68%) and 1 mL H_2O_2 (30%) at 130 °C for 12 min and then 160 °C for 50 min. After cooled to room temperature, the digested matter was adjusted to 50 mL using 1% HNO_3. Al^{3+}, P and K^+ were mesured with a inductive coupled plasma emission spectrometer (ICP) (ICP-OES, PerkinElmer, Avio™ 200).

2.7. Detection of chlorophyll contents and chlorophyll a fluorescence transient

Chlorophyll content was measured by soil and plant analyzer development instrument (SPAD-502, Minolta, Osaka, Japan). Chlorophyll a fluorescence transient was measured by a chlorophyll fluorometer (PAM 2500, Heinz Walz GmbH, Germany) with time resolution of 10 μs. Briefly, the chlorophyll a fluorescence (OJIP transient) was induced by strong light pulse which was 3000 photons μmol $m^{-1}s^{-1}$ after pretreated in dark for 30 min. The pulse was digitized between 10 μs and 320 ms. The JIP-test was conducted according to Strasser et al. (2010).

2.8. Analysis of ionic activity

Visual MINITEQ 3.1 was applied to simulate the phase and ionic activities of Al^{3+} and F^- in the solution. For parameter settings, The pH was set as "Calculated from mass balance", the ionic strength was set as "To be calculated", the concentration unit was set as "mg/L", the Temperature was set as "25 °C", the component names were Al^{3+} and F^-, Total concentrations were the concentrations of Al^{3+} and F^- in the prepared solution. Then add to list and run the program. The ions activities were recorded by the program.

2.9. Statistics analysis

Statistics analysis was based on one-way analysis of variance (ANOVA), Duncan's multiple range test with SPSS 16.0. $P < 0.05$ was considered as statistically significant. Data were showed as mean ±

standard deviation.

3. Results

3.1. Aluminium fluoride complexes in the solution of the treatments

The species in the treatment solution were different according to the components used in the experiment. When only aluminium was used, species in the solution included $Al^{3+}$and Al-OH complex such as $Al(OH)_2^+$, $AlOH^{2+}$, $Al(OH)_3$, $Al(OH)_4^-$, $Al_2(OH)_2^{4+}$ and $Al_3(OH)_4^{5+}$, and the contents of the different complex changed with additional amount of $Al_2(SO_4)_3$. In brief, contents of Al^{3+}, $Al(OH)_2^+$, $Al_2(OH)_2^{4+}$ and $Al_3(OH)_4^{5+}$ increased, however, $Al(OH)_3$ and $Al(OH)_4^-$ decreased with addition of $Al_2(SO_4)_3$. Besides, the highest content of $AlOH^{2+}$ was detected in the moderate concentration of $Al_2(SO_4)_3$, it decreased after addition of high concentration of $Al_2(SO_4)_3$. When only fluoride was used, species in the solution included F^-, HF, HF_2^-, and concentration of other species increased with the concentration of NaF. When aluminium and fluoride were used together, species in the solution included Al^{3+}, H-F complex, Al-OH complex, and Al-F complex (i.e. AlF^{2+}, AlF_2^+, AlF_3 and AlF_4^-). The contents of Al-OH complex decreased but Al-F complex increased after using of F. Moreover, AlF^{2+} content increased dramatically in high concentration of $Al_2(SO_4)_3$ combined with high concentration of NaF solution(Table 1).

3.2. Physiological changes of tall fescue cells under different treatments

Physiological changes of tall fescue were detected after it was treated with Al and F, the results were shown in Fig. 1. In detail, the plant biomass significantly decreased after Al4F5 and Al20F5 treatment. Compare to control, biomass decreased 33.2% and 33.3%, respectively, in the treatment of Al4F5 and Al20F5 (Fig. 1A). Simultaneously, chlorophyll content decreased significantly after different treatments. The highest chlorophyll content was detected in the plants of control, while the lowest was detected in the plants after Al20F5 treatment. Besides, if the plants were only treated with Al^{3+} or F^-, the chlorophyll content decreased gradually. Chlorophyll contents of tall fescue decreased 10.71% and 6.15%, respectively, in treatments of Al20F0 and Al0F5 compared to control (Fig. 1B). Accordingly, other physiological indexes, such as MDA content and relative EL, were also changed in tall fescue. The highest MDA content was detected in the plant after Al4F5 treatment, and it was 63.2% higher than that of control. Interestingly, the lowest MDA content was detected in the plants after treatment of Al0.4F0.5. Contrary to the change of chlorophyll contents, the MDA content increased in tall fescue after treatments of Al and F. The MDA contents in the treatments of Al20F0 and Al0F5 were 28% and 12.29% higher, respectively, than that of control (Fig. 1C). For the change of EL, it was showed that EL was highest in plants that after Al20F5 treatment, which was 1.69 fold higher than that of control. In general, the relative EL in tall fescue increased gradually with increase of Al^{3+} and F^- concentration. However, under Al0.4 treatment, realtive EL decreased after F^- addition. The realtive EL under treatment of Al0.4F5 was 26.31% lower than that of Al0.4F0 treatment (Fig. 1D).

3.3. Ion content changes in tall fescue after different treatments

Contents of aluminium, phosphorus (P) and potassium (K) were measured in the plants after different treatments. The results showed that aluminium contents were incresed remarkably with Al^{3+} concentration of the treatment solutions. Besides, when the plants were treated with same $Al_2(SO_4)_3$ concentration, the Al^{3+} content in tall fescue incresed with application of F^-. The highest Al content was observed in

Table 1
Aluminum and fluorine species of each treatment and their activities calculated from Visual Minteq 3.1.

Detailed species:	A0F0.5 activity (mol/L)	A0F5	A0.4F0	A0.4F0.5	A0.4F5	A4F0	A4F0.5	A4F5	A20F0	A20F0.5	A20F5
H^+	9.86×10^{-8}	3.46×10^{-11}	1.03×10^{-5}	2.51×10^{-6}	2.54×10^{-7}	3.48×10^{-5}	3.15×10^{-5}	4.42×10^{-6}	7.91×10^{-5}	7.76×10^{-5}	6.37×10^{-5}
Al^{3+}			4.33×10^{-9}	9.91×10^{-8}	1.96×10^{-11}	8.41×10^{-5}	6.77×10^{-5}	5.22×10^{-7}	4.81×10^{-4}	4.63×10^{-4}	3.07×10^{-4}
$Al(OH)_2^+$			2.34×10^{-6}	8.02×10^{-7}	1.55×10^{-8}	3.54×10^{-6}	3.47×10^{-6}	1.36×10^{-6}	3.91×10^{-6}	3.91×10^{-6}	3.85×10^{-6}
$AlOH^{2+}$			4.79×10^{-6}	3.98×10^{-7}	7.79×10^{-10}	8.41×10^{-5}	3.47×10^{-6}	1.19×10^{-6}	6.13×10^{-5}	6.01×10^{-5}	4.85×10^{-5}
$Al(OH)_3$ (aq)			9.10×10^{-8}	1.28×10^{-7}	2.45×10^{-8}	4.08×10^{-8}	4.41×10^{-8}	1.23×10^{-7}	1.99×10^{-8}	2.02×10^{-8}	2.43×10^{-8}
$Al(OH)_4^-$			4.33×10^{-9}	2.46×10^{-8}	4.75×10^{-8}	5.76×10^{-10}	6.87×10^{-10}	1.37×10^{-8}	1.23×10^{-10}	3.21×10^{-8}	1.87×10^{-10}
$Al_2(OH)_2^{4+}$			4.57×10^{-9}	3.16×10^{-11}	1.21×10^{-16}	1.18×10^{-7}	9.34×10^{-8}	2.83×10^{-10}	7.50×10^{-7}	7.21×10^{-7}	4.70×10^{-7}
$Al_3(OH)_4^{5+}$			1.35×10^{-10}	3.19×10^{-13}	2.36×10^{-20}	5.27×10^{-9}	4.08×10^{-9}	4.84×10^{-12}	3.70×10^{-8}	3.55×10^{-8}	2.28×10^{-8}
F^-	2.54×10^{-5}	2.60×10^{-4}		4.25×10^{-6}	2.14×10^{-4}		3.21×10^{-8}	6.50×10^{-6}		4.80×10^{-9}	6.91×10^{-8}
AlF^{2+}				4.31×10^{-6}	4.29×10^{-8}		2.23×10^{-5}	3.47×10^{-5}		2.27×10^{-5}	2.17×10^{-4}
AlF_2^+				7.62×10^{-6}	3.83×10^{-6}		2.98×10^{-7}	9.40×10^{-5}		4.54×10^{-8}	6.25×10^{-6}
AlF_3 (aq)				3.80×10^{-7}	9.64×10^{-6}		1.13×10^{-10}	7.17×10^{-6}		2.56×10^{-12}	5.07×10^{-9}
AlF_4^-				8.08×10^{-10}	1.03×10^{-6}		1.81×10^{-15}	2.33×10^{-8}		6.15×10^{-18}	1.76×10^{-13}
HF(aq)	3.79×10^{-9}	8.52×10^{-8}		1.61×10^{-8}	8.21×10^{-8}		1.53×10^{-9}	4.34×10^{-8}		5.63×10^{-10}	6.65×10^{-9}
HF_2^-	3.83×10^{-13}	3.35×10^{-8}		2.72×10^{-13}	7.00×10^{-11}		1.96×10^{-16}	1.12×10^{-12}		1.07×10^{-17}	1.83×10^{-15}
Classification											
Al-OH- complex	–	–	7.23×10^{-6}	1.35×10^{-6}	8.83×10^{-8}	8.78×10^{-5}	7.07×10^{-6}	2.69×10^{-6}	6.60×10^{-5}	6.48×10^{-5}	2.69×10^{-6}
Al-F- complex	–	–	–	1.23×10^{-5}	1.46×10^{-5}	–	2.25×10^{-5}	1.36×10^{-4}	–	2.28×10^{-5}	1.36×10^{-4}

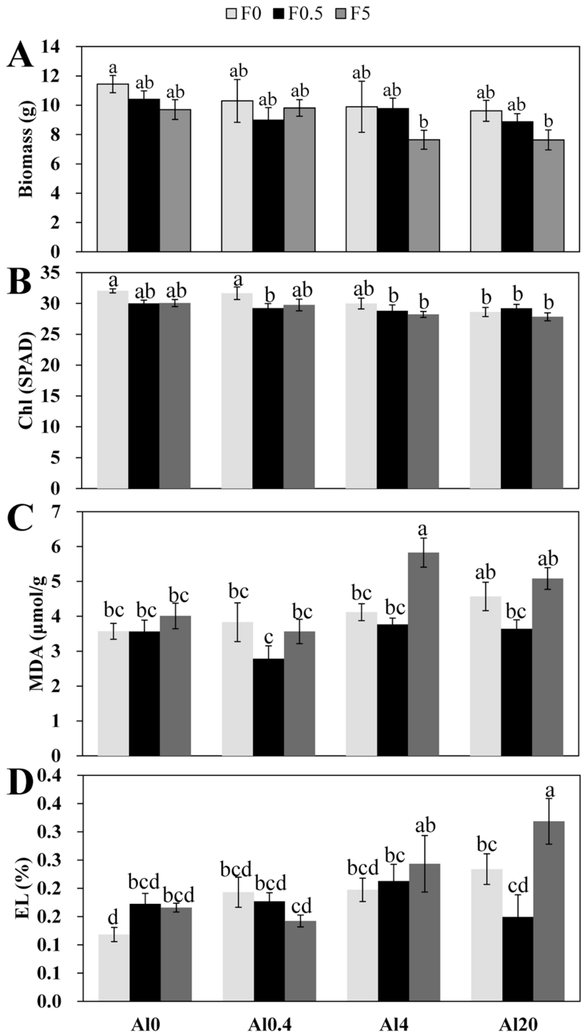

Fig. 1. Physiological changes in tall fescue after different treatments. (A) Biomass; (B) Chlorophyll content; (C) MDA content; (D) Relative EL.Al = $Al_2(SO_4)_3$ treatment; F = NaF treatment; numbers followed the letters were concentration of the solution.

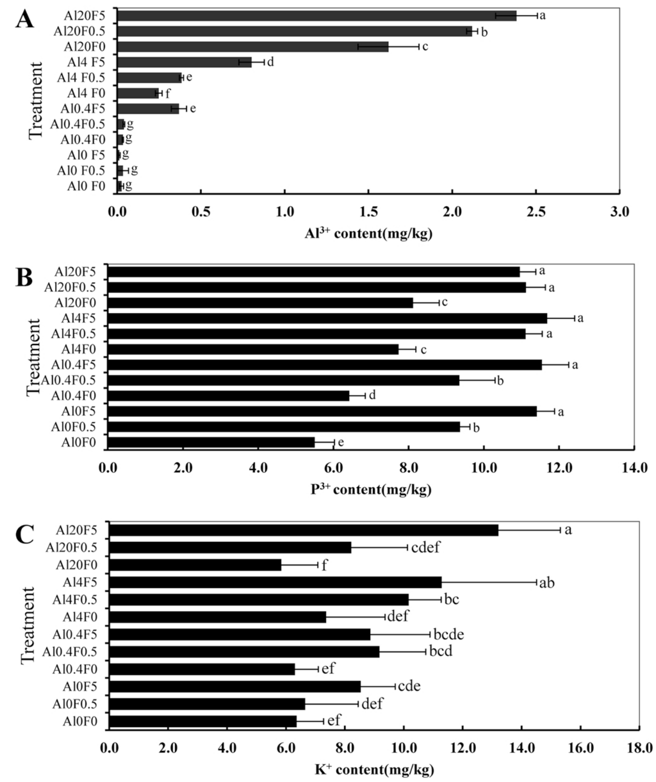

Fig. 2. Changes of ion content in tall fescue leaves after different treatments. (A) Al^{3+} content; (B) P^{3+} content; (C) K^+ content.Al = $Al_2(SO_4)_3$ treatment; F = NaF treatment; numbers followed the letters were concentration of the solution.

the plants of Al20F5 treatment, and it was 90.89 fold compared to control. Aluminium content of the plants that after Al0.4F0.5 treatment was even higher than that of Al4F0, although the concentration of Al^{3+} in Al0.4F0.5 was lower than that of Al4F0 (Fig. 2A). Similarly the P content in tall fescue increased with further application of Al^{3+}, and it also increased further use of F^-. The highest of P content detected in the plant after Al4F5 treatment, which was 2.12 fold compared to control (Fig. 2B). Moreover, the change of K content was also observed to increase with application of Al and F. The highest of K content was detected in the plant after Al20F5 treatment, which was 2.08 fold compared to control (Fig. 2C).

3.4. Changes of photosynthesis efficiency in tall fescue after different treatments

Photosynthesis is an important bioprocess in plant and it is very sensitive to stress conditions. To estimate the effects of Al^{3+} and F^- on photosystem of tall fescue, Chl *a* fluorescence was measured. The results showed that the OJIP transient of tall fescue declined significantly after Al^{3+} and F^- treatment, and combination of Al^{3+} and F^- treatment could aggravate the damage consequently (Fig. S1 and S2). The Chl *a* fluorescence transients were double normalized between F_0 and F_K with the formula of $W_{OK} = (F_t\text{-}F_0)/(F_K\text{-}F_0)$ (The basic parameters were shown in Table S1). ΔW_{OK} was calculated as transients of stress treated leaves minus that of control (Oukarroum et al., 2007). Interestingly, the results of ΔW_{OK} showed that when the concentration of Al^{3+} was constant, ΔW_{OK} increased dramatically after use of relative high concentration of F^-, and the peak was around 0.25 (Fig. 3). Besides, the most significant change of ΔW_{OK} was detected in the plants after Al20F5 treatment with a peak around 0.32 (Fig. 3D). Interestingly, it was showed that when the concentration of F^- was constant, ΔW_{OK} increased dramatically after addition of low concentration of Al^{3+}, but the variation amplitude after addition of high concentration of Al^{3+} was lower than that of low concentration of Al^{3+} (Fig. S3). To investigate the change of the photosynthetic behavior in tall fescue, JIP-test analysis was further conducted. The changes of photosynthetic parameters in tall fescue under different treatments showed that Al^{3+} and F^- could affect photosynthetic process in tall fescue which was revealed by JIP-test. In detail, when the plant was treated with 4 and 20 $mg\cdot L^{-1}$ φ_{P0} (Fv/Fm) decreased remarkably after using of F^-. The lowest φ_{P0} value was detected in the plant after Al20F5 treatment which was 95.4% of control (Fig. 4A). Similar result was also showed in δ_{R0}. As it was shown in the result, δ_{R0} was not affected by Al^{3+} treatment, however, it declined dramatically after addition of F^-. The lowest value of δ_{R0} was detected in the plant that after Al4F5 treatment which was 43.15% of control (Fig. 4C). It was interesting that the change of ψ_{E0} was different with that of φ_{P0} and δ_{R0}. Under a certain Al^{3+} concentration treatment, ψ_{E0} increased remarkably after application of F^-. The highest value of ψ_{E0} was detected in the plant that after Al0.4F5 treatment which was 56% higher than that of control (Fig. 4B). Besides, the results of PI_{ABS} showed that after relative low concentration (0.4 $mg\cdot L^{-1}$) of Al^{3+} treatment, PI_{ABS} increased slightly after application of F^-, while after high concentration (4 and 20 $mg\cdot L^{-1}$) of Al^{3+} treatment, it decreased with application of F, and the lowest value of PI_{ABS} was detected in the plant that after Al20F5 treatment which was 73.53% of control (Fig. 4D).

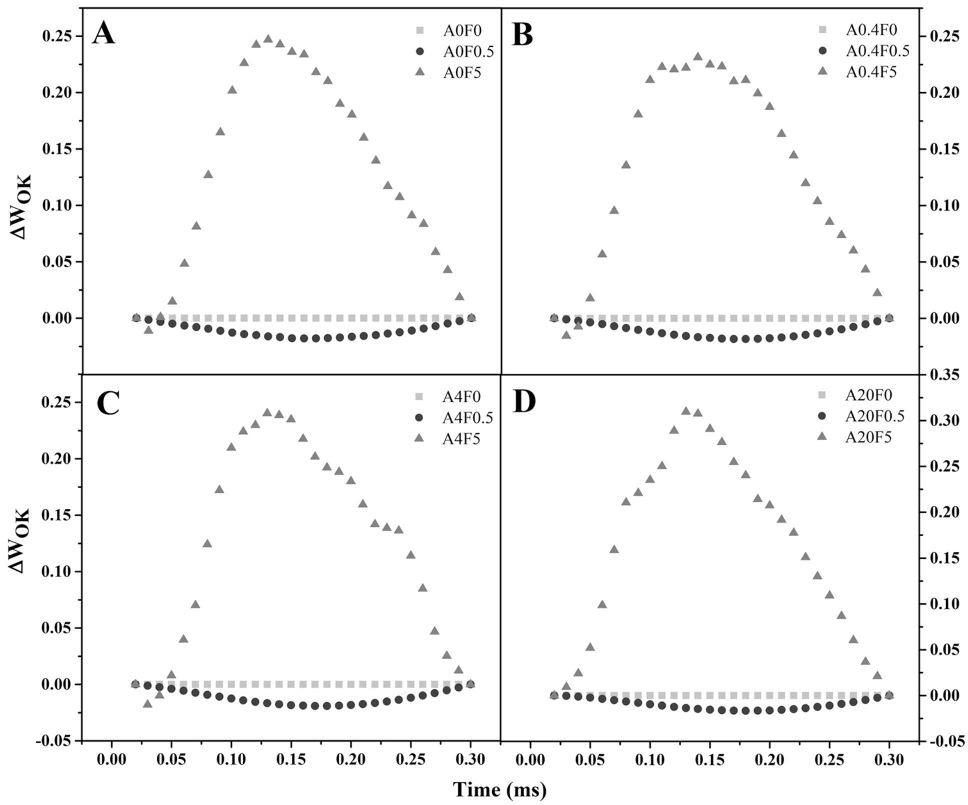

Fig. 3. Changes of L-band in tall fescue leaves after different treatments. (A) only NaF treatment; (B) 0.4 mg·L^{-1} $Al_2(SO_4)_3$ treatment; (C) 4 mg·L^{-1} $Al_2(SO_4)_3$ treatment; (D) 20 mg·L^{-1} $Al_2(SO_4)_3$ treatment.Al = $Al_2(SO_4)_3$ treatment; F = NaF treatment; numbers followed the letters were concentration of the solution.

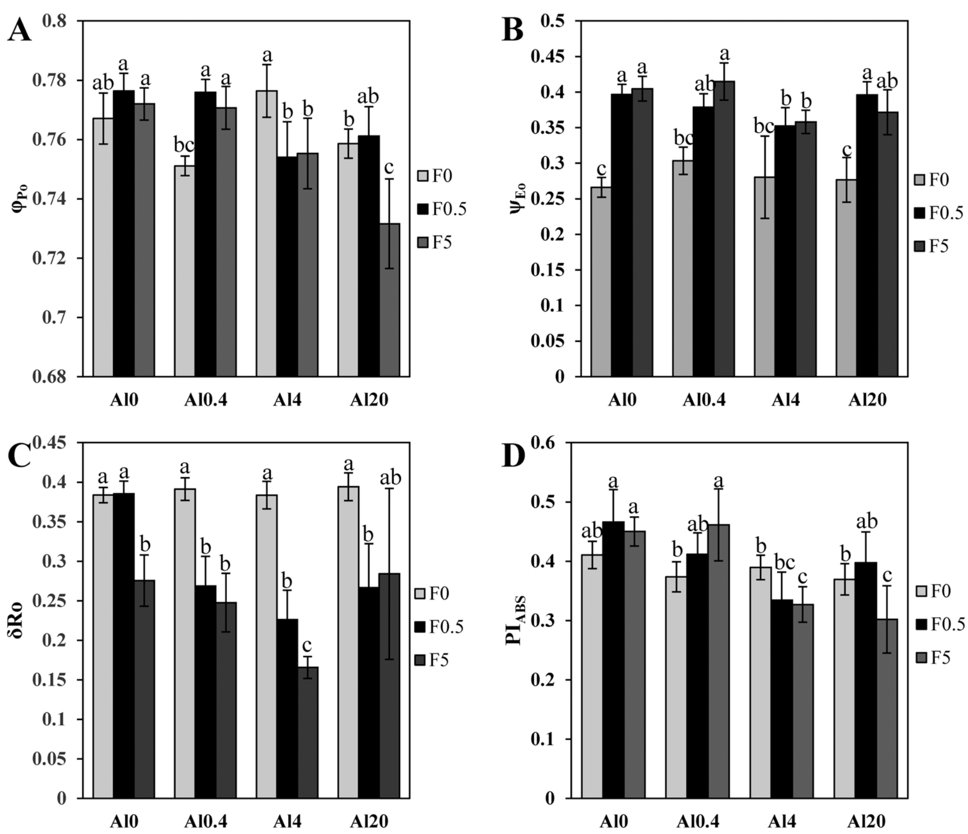

Fig. 4. Changes of photosynthesis efficiency parameters of tall fescue leaves after different treatments. (A) changes of φ_{P0}; (B) changes of ψE0; (C) changes of δ_{R0}; (D) changes of PI_{ABS}.Al = $Al_2(SO_4)_3$ treatment; F = NaF treatment; numbers followed the letters were concentration of the solution.

3.5. Correlation analysis between tall fescue physiological properties and aluminium fluoride complexes

The correlation between the detected parameters was shown in Table 2. As expected, MDA content and EL showed negative correlation with other parameters. According to the correlation coefficients, MDA content was negatively correlated with Chl significantly; and EL was negatively correlated with Chl, PI_{ABS} and φ_{P0} significantly. Interestingly, Chl was positively correlated with biomass and $PI_{ABS,}$ but negatively correlated with φ_{P0}. Correlation of physiological properties and aluminium fluoride complexes demonstrated that AlF^{2+} was positively correlated with EL and φ_{P0}. Generally, aluminium fluoride complex positively correlated with MDA and EL; but it negatively correlated with Chl and φ_{P0}. Among the all aluminium fluoride complexes, AlF^{2+} was positively correlated with EL and φPo, and AlF_2^+ had negative correlation with δRo.

4. Discussion

4.1. Physiological changes were induced by aluminium fluoride complexes in tall fescue

Aluminium is a major metallic element of most soils, and it is phytotoxic to many plants when the soil pH is below 5.5 (Schmitt et al., 2016). In common condition, Al exists as hydroxide form which is Al $(OH)_3$ in the soil. When it is exposed to acidic environment, $Al(OH)_3$ transforms to $Al(OH)_2^+$, $Al(OH)^{2+}$ and Al^{3+} which are toxic to plant (Kinraide, 1997). Because Al exists in the states of + 2 and + 3, it can easily form different compounds with negatively charged ions or ionic groups (Mossor-Pietraszewska, 2001). Besides, F^- is also phytotoxic to many plants, and complexes of Al-F predominated the F species in acidic soil (Ruan et al., 2003). It was also found that *CsXTH* genes were regulated by Al^{3+} and F^- in tea plants (Wu et al., 2021). The results of the present research showed that AlF^{2+}, AlF_2^+, AlF_3 and AlF_4^- were formed in the treatment solution when Al and F were combined together. This was in line with the results of previous study (Kinraide, 1997). In the present study, the concentration of H^+ was increased gradually with supplement of F which suggested increasing of the pH of the treatment solution. This was because NaF, a species of weak alkali, was used as F^- donor in this study. Consequently, the Al^{3+} content decreased remarkably with addition of F. It was reported that Al-F complexes were toxic to plants. For instance, the growth of oat (*Avena sativa*) and tomato (*Lycopersicon esculentum*) was limited by Al^{3+} and F^- treatment (Stevens et al., 1997). From this study, it was revealed that the biomass of tall fescue decreased after only Al^{3+} or only F^- treatment. But the change of MDA and EL were contrary to that of biomass. Moreover, it was also detected that the biomass of tall fescue was dramatically decreased after relative high concentration of Al^{3+} and F^- treatment. This implies that the cell membrane was damaged by the adverse condition, the growth and development was inhibited further. Interestingly, when the Al^{3+} concentration was constant, biomass of tall fescue decreased gradually with supplement of F^-. Contrary to biomass, the change of MDA and EL increased after supplement of Al^{3+} and F^-. This suggested that both Al^{3+} and F^- were toxic to tall fescue and more severe damages would be caused by combination of Al^{3+} and F^-. This seems inconsistent with previous study where Al^{3+} was reported to alleviate F^- toxicity in tea (*Camellia sinensis*) (Yang et al., 2016). This study revealed that MDA and EL of tall fescue decreased when treated with relatively low concentration of Al^{3+} ($0.4\ mg\cdot L^{-1}$) after supplement of F^-. This result implied that relatively low concentration of Al^{3+} contributed to the alleviation of F^- toxicity in tall fescue. Besides, when the Al^{3+} concentration was constant, relatively low concentration of F^- could decrease MDA and EL of tall fescue which suggested that alleviation of Al^{3+} induced some damages. However, when tall fescue was exposed to relatively higher concentrations of Al^{3+} and F^-, MDA and EL significantly increased. This results implied that high concentrations of Al^{3+} and F^- treatment were lethal to tall fescue. Hence, these results revealed that the toxic effects could be offset by low concentrations of Al^{3+} and F^-, but more severe damage would be induced by high concentrations of Al^{3+} and F^- combination solution. The reason was that at low concentration condition, toxic ions including Al^{3+} and F^- formed less toxic phases such as Al-OH complexes and Al-F complexes. However, at high concentration condition, although less toxic ion phases were formed, concentrations of toxic ions such as Al^{3+} and F^- were still high enough to induce lethal effect in tall fescue. These results suggested that low concentration of F^- could alleviate cell membrane damage induced by Al^{3+}. But when the concentration was above the threshold, it would induce more severe damage in cell membrane of tall fescue combined with Al^{3+}.

Table 2
Pearson correlation coefficients between tall fescue physiological properties and aluminum and fluorine species of each treatment.

	pH	MDA	Pro*	EL	Chl	Bio	PI_{ABS}	φpo	ψEo	δRo
MDA	-0.23									
Pro*	-0.52	0.73								
EL	-0.46	0.73	0.73							
Chl	0.57	-0.81*	-0.77	-0.96**						
Bio	0.37	-0.787	-0.83*	-0.75	0.84*					
PI_{ABS}	0.65	-0.75	-0.63	-0.91*	0.96**	0.66				
φpo	0.63	-0.70	-0.83*	-0.88*	-0.85*	0.60	0.86*			
ψEo	0.44	-0.47	-0.11	-0.64	0.71	0.37	0.82*	0.43		
δRo	-0.27	-0.55	0.14	-0.04	0.18	0.15	0.24	-0.08	0.45	
Al^{3+}	-0.77	0.05	0.60	0.12	-0.21	-0.26	-0.18	-0.44	0.21	0.54
$AlOH^{2+}$	-0.50	-0.21	0.14	-0.43	0.24	0.06	0.23	0.11	0.36	0.26
$Al(OH)_2^+$	0.98 * *	0.21	0.52	0.43	-0.51	-0.25	-0.61	-0.68	-0.36	0.31
F^-	0.79	-0.23	-0.30	-0.48	0.62	0.51	0.65	0.35	0.72	-0.01
AlF^{2+}	-0.52	0.53	0.86*	0.87*	-0.79	-0.66	-0.70	0.90*	-0.24	0.38
AlF_2^+	0.18	0.75	0.23	0.29	-0.45	-0.61	-0.37	-0.08	-0.43	-0.85*
AlF_3 (aq)	0.81	0.27	-0.13	-0.26	0.28	0.09	0.35	0.27	0.36	-0.57
AlF_4^-	0.78	-0.22	-0.29	-0.47	0.62	0.51	0.64	0.34	0.72	-0.01
$Al(OH)_3$ (aq)	0.32	0.09	-0.28	0.05	-0.12	-0.29	-0.04	0.41	-0.43	-0.48
$Al(OH)_4^-$	0.98 * *	-0.33	-0.49	-0.53	0.66	0.42	0.75	0.63	0.62	-0.09
HF	0.90*	0.08	-0.22	-0.33	0.41	0.21	0.49	0.36	0.50	-0.37
Al-OH complex	-0.77	0.13	0.69	0.23	-0.30	-0.36	-0.25	-0.52	0.18	0.56
Al-F complex	-0.37	0.83*	0.90*	0.93**	-0.91*	-0.87*	-0.79	-0.87*	-0.39	-0.03

* Correlation is significant at the 0.05 level (2-tailed);
** Correlation is significant at the 0.01 level (2-tailed).

4.2. Photosystem of tall fescue was interrupted by aluminium fluoride complexes

Chlorophyll is an important component in plant and is very sensitive to stress conditions. Chlorophyll degradation massively occurring under biotic and abiotic stresses (Hu et al., 2014). The results of this study revealed the chlorophyll content decreased after Al^{3+} and F^- treatment especially high concentration of Al^{3+} and F^- combination treatment. The degradation of chlorophyll would induce cell senescence further which leaf chlorosis. Hence, the decrease of chlorophyll content suggested physiological damage induced by different treatments in tall fescue. Change of chlorophyll would inevitably induce change of photosynthesis process. In the photosynthetic apparatus, photosystem II (PSII) was very sensitive to environmental stresses (Baker, 2008). To estimate the change of photosynthesis process at different treatments in tall fescue, chlorophyll *a* fluorescence analysis was exploited (Strasser et al., 2010). The results revealed changes of photosynthesis related parameters after different treatments. It was showed that φ_{P0} decreased after Al^{3+} and F^- treatment in tall fescue. φ_{P0}, also be known as F_V/F_M, reflected the maximum quantum yield for primary photochemistry, it was widely used to assess the stresses tolerance of plants (Zhang et al., 2016). Decrease of φ_{P0} implied the decrease of maximum quantum efficiency of PSII, and it suggested the photosynthetic efficiency of PSII was negatively affected by adverse conditions. For example, F_V/F_M was used for heat tolerant cultivar selection of wheat (*Triticum aestivum*) (Sharma et al., 2015). ψ_{E0} reflected the efficiency that an electron was transferred further than Q_A^-. Interestingly, ψ_{E0} increased remarkably in tall fescue after treated with F^-, this revealed that F^- was involved in improving the activity of electron transport beyond Q_A. δ_{R0} reflected the efficiency of an electron was transferred from the intersystem electron carriers to the acceptors at the PSI acceptor side. The present results showed that δ_{R0} dramatically decreased in tall fescue after F^- treatment. PI_{ABS} was performance index of the energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors (Strasser et al., 2010). This study showed that the PI_{ABS} in tall fescue increased slightly after F^- treatment under low concentration of Al^{3+} stress. But it significantly decreased when exposed to F^- under relative high concentration of Al^{3+} stress. These results suggested that the effect of photosynthesis induced by Al^{3+} and F^- in tall fescue was complicated. Photosynthesis parameters were improved when treated with low concentration of Al^{3+} and F^- combined solution compared to that of only Al^{3+} or F^- treatment. However, the parameters were negatively affected when treated with relative high concentration of Al^{3+} and F^- combined solution. In the previous study, it was reported that photosynthetic pigments of soybean (*Glycine max*) and photosynthesis of tea (*Camellia sinensis*) were negatively affacted by Al^{3+} and F^- stress (Milivojević et al., 2000; Cai et al., 2016). Considering that F^- was involved in aluminium detoxification in plant (MaClean et al., 1992), maybe this was the reason why the photosynthetic parameters were improved by low concentration of F^- in tall fescue. But when tall fescue was treated with relative high concentration of F^-, the photosynthetic parameters were significantly inhibited. It was reported that, Al^{3+} could improve the uptake of F^- in tea plant (*Camellia sinensis*) (Ruan et al., 2003). The excessive F could induce cell damage in plant (Li et al., 2011). ΔW_{OK}, also known as L-band, was different between normalized stress induced transients and control (Chen et al., 2013) and the difference made L-band visible. The negative amplitude of L-band implied the increase of the energetic connectivity and vice versa. Grouping was sensitive to staching of thylakoid membranes, and the decreased L-band suggested improvement of the ability to resist stress induced changes in the structure/stacking of the thylakoid membranes (Oukarroum et al., 2007). So, the results of ΔW_{OK} in tall fescue suggested that low concentration of F^- could induce improvement of energetic connectivity, but relatively higher concentration of F^- induced decrease of energetic connectivity in tall fescue. Hence, the results of the present study suggested that low concentration of F^- played positive role in improving photosynthetic efficiency of tall fescue under Al^{3+} stress. While, relatively higher concentration of Al^{3+} and F^- combination treatment could inhibit the photosynthesis process in tall fescue. Theses results were also in line with that of MDA and EL.

4.3. Correlation analysis between tall fescue physiological properties and aluminium fluoride complexes

Correlation analysis was widely applied to investigate environmental stress response in plants (Glauser et al., 2010). It was used to study the cold response in oil palm (*Elaeis guineensis*) (Li et al., 2019) and traffic stress response in seashore paspalum (*Paspalum vaginatum*) (Jiang et al., 2003). According to the results of correlation analysis, the content of aluminium fluoride complexes, including AlF^{2+} and AlF_2^+, were significantly correlated to several physiological indexes, such as MDA content, EL, chlorophyll content, and φ_{P0}, of tall fescue. This implied that Al-F complex played very important roles in Al^{3+} and F^- combined induced toxicity in tall fescue. Kinraide had reported that AlF^{2+} and AlF_2^+ were toxic to plant despite that their toxicity was less than Al^{3+} (Kinraide, 1997). Besides, MDA content and EL were negatively correlated with other physiological indexes. This was reasonable because these two indexes reflected the damage of cell membrane, if the cell membrane was severely damaged the MDA content and EL would incresed dramatically while other physiological indexes were negatively affected. Moreover, AlF^{2+} content increased remarkably after combination of high concentration of Al^{3+} and F^- treatment. Considering that negative effect was distinctly measured in tall fescue after high concentration of Al^{3+} and F^- treatment, and AlF^{2+} content was significantly associated with EL and φP_O, hence AlF^{2+} might play an important role in inducing toxic effect in tall fescue. This phenomenon suggested that AlF^{2+} might have strong toxicity to the plant, and it induced toxic effect in plant through interfering photosynthesis process of the plant.

4.4. Nutrients uptake was hindered by aluminium fluoride complexes

It was reported that nutrient uptake of plant was affected by aluminium, the K^+ concentration increased with Al^{3+}, while Ca^{2+} and Mg^{2+} concentrations decreased in *Eucalyptus mannifera* and *Pinus radiata* (Huang and Bachelard, 1993). The nutrient uptake was also changed in tall fescue after treatment of Al^{3+} and F^- which was showed in the present study. As expect, the Al^{3+} concentration in tall fescue leaves dramatically increased after treated with Al solution, and the Al uptake enhanced with increase of Al^{3+} concentration in treatment solution. Besides, the exploit of F^- enhanced Al^{3+} uptake into tall fescue further. Simultaneously, similar changes were observed in P and K uptake. Previous research had reported that nutrient uptake increased significantly in rice when it was exposed to the stress of Al^{3+} combined with P (Hai et al., 1989). So, the results obtained in tall fescue were in accordance with that in rice. It was considered that Al^{3+} toxicity in plants probably depend on the balance of Al^{3+} with other elements (Huang and Bachelard, 1993). The increase of other nutrients maybe because of amelioration effects of F^-, however the real reason needs to be investigated further.

5. Conclusion

The results of this study indicated that high concentration of Al^{3+} and F^- could induce severe damage in growth and development of tall fescue. Cell membrane stability was injured, photosystem activity was inhibited, and nutrients uptake was interrupted in tall fescue after Al^{3+} and F^- treatment. Meanwhile, low concentration of F^- could alleviate physiological damages that induced by Al^{3+} in tall fescue. This was maybe because of the various aluminium fluoride complexes in Al^{3+} and F^- combined solution. Among the different complexes, AlF^{2+} was found as the most toxic compound to tall fescue.

CRediT authorship contribution statement

Jibiao Fan: Writing – original draft. **Ke Chen:** Experiment design, Supervision. **Jilei Xu:** Methodology, Investigation, Software. **Khaldun ABM:** Writing – review & editing. **Yao Chen:** Investigation. **Liang Chen:** Data curation. **Xuebing Yan:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113192.

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Further reading

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