Winter wheat adapts to environmental pH by changing H⁺ net flux in roots at the seedling stage

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Abstract

Changes in rhizosphere pH play an important role in wheat growth. To investigate the relationship between changes in rhizosphere pH and the growth of winter wheat roots and to explore the regulatory mechanism of acid and alkali resistance in winter wheat roots, the semi-winter wheat varieties Aikang 58 (AK58) and Bainong 4199 (BN4199) were used as materials for hydroponic experiments. Three pH levels (4.0, 6.5, and 9.0, with 6.5 as control) were applied during the wheat seedling stage. The results showed that the shoot and root biomass of the plants significantly decreased compared with the control under acid-base stress, with a more significant decrease with acid stress than alkali stress. Compared with the control, the root/shoot ratio increased under alkali stress and decreased under acid stress. The wheat root system showed H⁺ net efflux at pH 6.5 and 9.0, and the H⁺ net efflux rate at pH 4.0 was significantly lower

than the control. The root activity of wheat was higher than the control at pH 9.0 and lower at pH 4.0. The change of root pH was showed pH 4.0 < pH 6.5 < pH 9.0. Correlation analysis showed that changes in H⁺ net flux were significantly positively correlated to root activity and root pH. The H⁺ efflux rate and root activity of BN4199 were higher than AK58 under acid and alkali stress, and the root/shoot ratio was relatively high, indicating strong acid and alkali resistance. We conclude that wheat could adapt to poor acid-base environments by adjusting root H⁺ net flux, and in practice, the root/shoot ratio could be used as index for the rapid determination of acid-base tolerance in wheat at the seedling stage.

Keywords: Wheat · H⁺ net flux · Root activity · Root pH · Root/shoot ratio

Introduction

Wheat (*Triticum aestivum* L.) is an important cereal crop globally, and its growth and yield formation are largely affected by soil environmental conditions [1,2], particularly the recent intensification of soil acidification, which has severely affected food production and sustainable agricultural development [3,4]. The optimal soil pH for wheat growth is 6.5–7.0, and excessively acidic or alkaline soil may restrict the growth and development of wheat. Soil pH can affect the absorption of different ions by crops [5], thus affecting the overall growth status of wheat. Although reasonable water and soil management and chemical regulation play important roles in improving soil chemical properties [6], large-scale application improvements are relatively time-consuming and costly [7,8]. Therefore, cultivating acid- and alkali-tolerant wheat varieties is the most economical and effective method in addressing wheat propagation in acidic or alkaline soil.

Soil pH is largely influenced by the H⁺ concentration in the soil solution. In wheat production, the root system is in direct contact with the soil environment and is an important organ for the absorption and transport water and nutrients [9]. Hinsinger et al. [10] suggested that root-induced proton efflux or influx could respectively result in a significant decrease or increase in rhizosphere pH, usually up to ± 0.1 to 1 pH

unit, and in some cases as high as $\pm 2-3$ pH unit. Cu et al. [11] showed that wheat monocultures raised soil pH by 0.8 unit, while lupin monoculture lowered it by 0.3 pH unit, and mixed cultures result in soil pH that is intermediate of the two monocultures. In a strongly acidic, Cu-contaminated soil, the rhizosphere pH of wheat increased over 6 mm in soil, reaching up to +2.8 pH units close to the root mat surface [11]. Root-induced changes in rhizosphere pH will change the availability of nutrients in soil [12], thereby affecting plant growth and development. At the rhizosphere range, it has been demonstrated that protons are released by roots to compensate for the excessive absorption of cations by plants over anions [13]. The concentration of protons determines the pH of the solution, and H^+ -ATPase activity helps maintain the pH of cells and provides an H^+ electrochemical gradient for the influx or efflux of ions into or from the cells [14]. Xue et al. [15] suggested that enhancing the vacuolar Na^+/H^+ antiporter protein levels could improve salt tolerance in wheat in saline soils. Bouthour et al. [16] suggested that the NADPH circulatory system is associated with enhanced salt tolerance in wheat. In addition, soil acidification may cause soil aluminum (Al) toxicity and heavy metal pollution, which will cause active Al toxicity in wheat [17] as well as Cu [18], Fe [19], Mn [20], Pb [21], Cr [22], and other heavy metal poisoning. In addition, soil salinization can cause damage to wheat such as water stress, ion toxicity, and oxidative stress, thus affecting growth [23]. Borzouei et al. [24] found that wheat varieties with strong tolerance have better growth characteristics, and the yield of tolerant wheat varieties is higher than that of sensitive varieties [25]. Therefore, it is of great significance to select acid-alkali-resistant wheat varieties for stress tolerance breeding.

Wherrett et al. [26] found that the rhizosphere environment of wheat would affect changes in root H^+ net flux. However, studies on changes in H^+ net flux in wheat roots under different pH conditions are limited. Because roots are the first plant parts that respond to stress, it is highly relevant to study the effect of different pH levels on wheat growth using roots, including its underlying mechanism of resistance to acid and alkali stress. Therefore, hydroponics was used to simulate different rhizosphere pH environments, analyze biomass, root/shoot ratio, root H^+ net flux, root

activity, and root pH of winter wheat and to explore the root regulatory mechanism of wheat to adapt to different rhizosphere pH levels to provide a reference for identifying acid- and alkali-resistant varieties and yield improvement.

Materials and methods

Plant materials and treatment

Two winter wheat (*Triticum aestivum* L.) varieties, Aikang58 (AK58) and Bainong4199 (BN4199), which are widely cultivated in the North China Plain, were used in this study. Wheat seeds were surface-sterilized with 1% H₂O₂ for 24 h, and then rinsed well with distilled water. The presoaked seeds were placed in culture dishes. After full emergence, the wheat seedlings of uniform size were transplanted to Hoagland's solution that consisted of the following: 5 mM NH₄NO₃, 0.75 mM MgSO₄, 0.5 mM KH₂PO₄, 0.072 mM Fe-EDTA, 0.1 mM Na₂SiO₃, 50 μM KCl, 10 μM MnSO₄, 50 μM H₃BO₃, 2 μM ZnSO₄, 1.5 μM CuSO₄, and 0.075 μM (NH₄)₆Mo₇O₂₄. After 3 days of growth in nutrient solution (pH 6.5), 3 pH levels of nutrient solution were prepared, namely, pH 4.0 (acid stress), pH 6.5 (normal environment as control), and pH 9.0 (alkaline stress); the pH of the solution was adjusted with 1 mol/L HCL and 1 mol/L NaOH. Approximately 10–20 seedlings were measured for each indicator following a completely randomized design. Plants were grown in a growth chamber with a 14-h photoperiod (light intensity 20,000 lux), day/night temperatures of 22°C/19°C, and relative humidity of 64%. The cultivation boxes were randomly moved daily to minimize position effects. The nutrient solution was replaced at the same time every day. Ten-day-old wheat seedlings (second true leaf folded) were selected to monitor net fluxes of H⁺ in roots in response to different rhizosphere pH, and seedlings that were cultured for 15 days were selected for assessment of other indexes.

Dry matter accumulation

Dry matter accumulation in the roots and shoots at different pH levels was measured by the oven-drying method at 105°C for 30 min and then at 80°C until a constant weight was achieved to determine dry matter. The roots were soaked in 20 mM Na₂-EDTA for 10 min to remove any metal ions adhering to the root surface

before drying.

Net H⁺ flux at the root surface

To measure H⁺ flux, measuring solution (0.5 mM NH₄NO₃, 0.1 mM CaCl₂, 0.3 mM MES,) with different pH levels were prepared by adjusting the pH with HCl (acidic ingredient) or Tris (alkaline ingredient). This experiment adopted the instantaneous replacement method (quickly replacing testing solutions with different pH during the measurement to change the test pH environment of the material) to measure the absorption kinetics of H⁺ flux at different acid-base levels. Net H⁺ flux was measured using the non-invasive micro-test technology (NMT) (BIO-001A, Younger USA Sci. & Tech. Co., Amherst, MA, USA). The principle behind this method and the instrument used have been previously described [27,28]. Electrodes, holder, and LIX were provided by Xuyue Science & Technology Co. (Beijing, China) (<http://www.xuyue.net>). The data on H⁺ flux were calculated using MageFlux (Younger USA Corp.; <http://youngerusa.com/mageflux>).

Microelectrodes were calibrated with different concentrations of H⁺ ion buffers before measurement; electrodes with a slope of -58±2 for measuring H⁺ flux were used in this study. The main radicle, about 3 cm from the root tip, was cut to determine the maximum absorption sites of H⁺ in at least five similar fine roots [29], the maximum ion flux occurred within the region 0 to 15 mm from the root apex. Thus, we selected this region for measurements, balancing 10 min in the measuring solution of pH 6.5 to eliminate local ion imbalances, and then replacing with a new measuring solution for measurement. Next, a blank experiment without plant material was conducted before each formal test to eliminate the influence of the electrode motion agitation of the solution on the ion fluxes [30]. The H⁺ gradient near the root surface (about 5µm above the root surface) was measured by moving the ion-selective microelectrode between two positions separated by a distance of 30 µm in a direction perpendicular to the root axis. To investigate net H⁺ flux at different pH levels, first, ion flux was measured at pH 6.5 for 5 min, and then the test solution was immediately changed to pH 4.0 or pH 9.0 for 10 min. Each treatment was repeated using eight plants, the abnormal data set was removed, and the final calculated ion data were the

average of five replicates. The recording rate for the ion flux was one reading per 4 s.

Root activity

Root activity was assessed using the TTC reduction method. Fifteen seedlings were selected for each treatment, the roots were washed with distilled water, and then the roots were cut to 1-cm-sized cubes and mixed well. Approximately 0.5 g of roots was weighed and placed into a 15-mL centrifuge tube. Each treatment was repeated thrice, 5 mL of phosphoric acid buffer at pH 7.0 and 5 mL of 0.4% TTC were added in succession and kept in the dark at 37°C for 3 h. After incubation, 2 mL of 1 mol/L sulfuric acid were added to stop the reaction. After filtration, 10 mL of methanol were added and incubated at 30°C–40°C for 1–2 h until the root tips were completely white. The OD value was measured at a wavelength of 485 nm using a spectrophotometer.

Root pH

The roots were cut off and washed with distilled water, and absorbent paper was used to absorb moisture from the root surface. Two grams of fresh roots were ground, and each treatment was repeated thrice. Approximately 4 mL of distilled water were then added and mixed on an oscillator, left to stand at room temperature for 20 min, centrifuged at 12,000 rpm for 15 min, the supernatant was collected and used in measuring pH with a pH meter.

Data processing

The data shown were analyzed by standard ANOVA assumptions of homogeneity using SPSS 19.0 (Statistical Product and Service Solutions, IBM) and mapping by SigmaPlot 12.0 (Systat Software, Inc., Dundas, CA, USA). Statistical analysis of data was done according to LSD test ($p < 0.05$). The relationship between H^+ net flux and root characteristics was assessed by linear correlation analysis.

Results

Dry matter accumulation

Under different pH conditions, root dry weight was significantly different between the two cultivars, but the difference in shoot dry weight was not significant (Fig 1). The shoot and root biomass of the two cultivars could be arranged in

decreasing order as follows: pH 6.5 > pH 9.0 > pH 4.0. The root dry weight of BN4199 was significantly higher than AK58 under different pH conditions, and shoot dry weight was higher than AK58, but the difference was not significant. The root/shoot ratio of the two varieties increased compared with the control at pH 9.0 and decreased compared with the control at pH 4.0. There was no significant difference in the root/shoot ratio between the two cultivars at pH 6.5, and the root/shoot ratio of BN4199 was significantly greater than AK58 at pH 4.0 and 9.0.

H⁺ net flux

The H⁺ net flux of both cultivars showed efflux at pH 6.5 (Fig 2B). At pH 4.0, the H⁺ net flux of the two varieties significantly decreased compared with the control, whereas H⁺ net flux slowly decreased with time, and the H⁺ net efflux rate approached zero (Fig 2A). At pH 9.0, the instantaneous performance showed a large H⁺ net influx and gradually showed H⁺ net efflux over time (Fig 2C). The change trend of the two wheat varieties at different pH levels was the same. The H⁺ efflux rate of BN4199 was significantly lower than AK58 at pH 6.5 and significantly higher than AK58 at pH 4.0 and 9.0 (Fig 2D).

Root activity

The root activity of the two wheat varieties increased with higher pH (Fig 3). There was no significant difference in root activity between the two varieties at pH 6.5 and 9.0, and the root activity of BN4199 was significantly higher than AK58 at pH 4.0.

Root pH

The root pH of the two varieties was significantly different with various pH levels (Fig 4). The change trend of root pH of the two varieties was similar under different pH conditions and both increased in the following order: pH 4.0 < pH 6.5 < pH 9.0. The root pH of BN4199 was lower than AK58 at pH 6.5 and higher than AK58 at pH 4.0, but the difference was not significant. The root pH of BN4199 was significantly lower than AK58 at pH 9.0. Root pH in BN4199 and AK58 decreased by 1.92% and 2.70% respectively compared to the control at pH 4.0 and increased by 3.99% and 4.15% respectively compared to the control at pH 9.0, indicating that the

pH change range of BN4199 was smaller than AK58 under acid and alkali stress.

Correlation analysis

Table 1 shows that correlation analysis revealed that shoot and root biomass accumulation was positively but not significantly correlated with changes in H^+ net flux, root activity, and root pH. The change in H^+ net flux was significantly positively correlated with root activity and root pH.

Discussion

Rhizosphere pH can affect the absorption and utilization of mineral nutrients by wheat roots [31], thereby affecting the morphology and growth of wheat roots. This study has determined that biomass accumulation in the shoots and roots of wheat was highest at neutral pH, acid-base stress inhibited root and shoot growth, especially under acid stress, and biomass accumulation in wheat roots significantly decreased, while root biomass slightly decreased under alkali stress, and biomass accumulation in shoots was less affected by acid and alkali stress (Fig 1). The accumulation of shoot and root biomass changed the size of the root/shoot ratio of the plant. Under acidic stress, root biomass decreased more than the shoot, and root/shoot ratio decreased. Under alkaline stress, root biomass decreases less than the shoot, thus the root/shoot ratio increased (Fig 1). The root/shoot ratio shows the distribution of assimilation between the aboveground and underground organs. Changes in root/shoot ratio are a common response of plants in response to different stresses [32]. Dry matter accumulation and root/shoot ratio of plants under alkali stress were greater than that under acid stress, suggesting that maintaining a higher root/shoot ratio under acid and alkali stress is beneficial to the accumulation of total plant biomass (Fig 1). Compared with the two varieties, BN4199 has a higher root growth rate and root/shoot ratio under acid alkali stress (Fig 1).

The wheat root system is an active absorbing organ, and the strength of root activity reflects the metabolic ability of the root system, which directly influences the growth of aboveground parts [33]. This study has determined that the root activity of wheat remained high at pH 9.0, whereas at pH 4.0, root respiration was weakened,

dehydrogenase activity decreased, thus root activity decreased (Fig 3). H^+ ion concentrations influence the growth of plant roots and soil microorganisms, whereas the root system can release H^+ or OH^- to compensate for the imbalance in anion and cation absorption at the soil-root interface [10]. The release or absorption of H^+ by roots changes the rhizosphere pH, thereby influencing nutrient availability [34]. The results of the present showed that the H^+ efflux rate of wheat roots was significantly higher than that of the control at pH 9.0. H^+ net efflux acidified the rhizosphere and lowered the pH level of the rhizosphere, which could improve the availability of nutrients in the rhizosphere solution, thereby promoting root growth and increasing the root/shoot ratio (Fig 1 and 2). Oliva et al. [35] suggested that proton efflux under low pH conditions avoided the accumulation of toxic H^+ in the cytoplasm and supported more nutrient acquisition by H^+ -tolerant species. This study found that the H^+ efflux rate was significantly lower than that of the control at pH 4.0, root activity decreased, root growth was inhibited, and root/shoot ratio decreased, further indicating that acid stress inhibited the net release of H^+ , and maintaining a high net H^+ release under acidic condition stabilizes cytoplasmic pH (Fig 1–3). Compared with AK58, BN4199 maintained a higher H^+ efflux rate and root activity under acid and alkali stress (Fig 2 and 3).

Correlation analysis showed that there was a significant positive correlation between changes in H^+ net flux and root activity, indicating that root activity adjusted H^+ net flux changes, and the increase in root activity accelerates cellular redox reactions, increases dehydrogenase activity, and stimulates H^+ efflux (Table 1). Changes in H^+ net flux will affect the pH of root cells [36], thereby influencing root pH homeostasis and metabolic pathways [37,38]. Correlation analysis also showed that H^+ net flux was significantly positively correlated with changes in root pH, indicating that changes in H^+ net flux regulate root pH, thereby affecting the metabolic activity of root cells (Table 1). Li et al. [39] believed that the efficiency of the intracellular pH regulatory system before and after stress reflects the degree of plant resistance to stress. Compared with the control, the root pH decreased under acidic conditions, while increased under alkaline conditions. Under acidic conditions,

the passive influx of H^+ acidified root pH, the H^+ efflux rate of BN4199 was higher than that of AK58, which resulted in root pH alkaline (Fig 2 and 4). The H^+ efflux rate of BN4199 was higher than AK58 under alkaline conditions, consequently rhizosphere acidification reduced root pH, and the acid-base balance inside and outside the cells promoted root growth (Fig 2 and 4). In terms of root pH, this was close to neutral regardless of whether the rhizosphere was acidic or alkaline (Fig 4). This may be a regulatory mechanism for the root system to adapt to the acid-base environment, which needs further investigation.

The effect of rhizosphere pH on wheat varied with variety. Gao et al. [41] considered that the accumulation of biomass reflected the response of plants to stress. Under acid-base stress, biomass accumulation, H^+ net flux, and root activity of BN4199 were all greater than AK58, and root pH tended to be neutral, which indicated that BN4199 has a strong ability to autonomously adjust root pH under acid-base stress (Fig 1–4). Terletskeya et al. [42] earlier reported that the root/shoot ratio of more tolerant wheat varieties increased with stress, whereas that of sensitive varieties decreased with stress. The root/shoot ratio of BN4199 was significantly higher than AK58 under acid-base conditions, and there was no significant difference between the two varieties under neutral conditions, indicating that BN4199 has stronger acid and alkali resistance than AK58 (Fig 1). Therefore, in wheat ecological breeding, the ability of wheat seedlings to tolerate acid and alkali can be quickly identified by measuring the root/shoot ratio.

Conclusions

Wheat could adapt to poor acid-base environments by adjusting root H^+ net flux, and wheat cultivars under different rhizosphere pH values in our study showed variations in acid and alkali tolerance-related traits at the seedling stage. Wheat varieties with strong acid and alkali resistance have high H^+ efflux rates, strong root activity, and neutral root pH. Root H^+ efflux under alkali stress acidifies the rhizosphere, which is beneficial to root growth and increases root/shoot ratio. In practice, the root/shoot ratio could be used as index for the rapid determination of

acid-base tolerance in wheat at the seedling stage.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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Fig. 1 Root and shoot dry weigh of AK58 and BN4199 seedlings after 15 d of treatment at different pH levels. Results are shown as means $\pm$ SD. Vertical bars indicate the standard deviation, and different letters above the error bars indicate significant differences among pH levels between wheat cultivars at $P < 0.05$ using the LSD test.

Fig. 2 Influence of pH on H⁺ net flux in wheat root surfaces. Transient H⁺ kinetics of different pH levels in AK58 and BN4199 roots were measured in root epidermal cells; the dynamic changes in wheat root H⁺ net fluxes (about 600 s) are presented after changing acidic test liquid (A) or alkaline

test liquid (C). The mean $\pm$ SE of H^+ net fluxes during the measurement period are shown ($n = 5$). B shows changes in H^+ net flux after 5 min at pH 6.5. D shows the mean flux of each treatment 1 min before the end of measurement. Vertical bars indicate the standard deviation, and different letters above the error bars indicate significant differences among pH levels between wheat cultivars at $P < 0.05$ using the LSD test.

Fig. 3 Root activity of AK58 and BN4199 seedlings after 15 d of treatment at different pH levels. Results are shown as means $\pm$ SD. Vertical bars indicate the standard deviation, and different letters above the error bars indicate significant differences among pH values between wheat cultivars at $P < 0.05$ using the LSD test.

Fig. 4 The root pH of AK58 and BN4199 seedlings after 15 d of treatment at different pH levels. Results are shown as means $\pm$ SD. Vertical bars indicate the standard deviation, and different letters above the error bars indicate significant differences among pH levels between wheat cultivars at $P < 0.05$ using the LSD test.

Table 1 Correlations between root and shoot weight, H^+ net flux, and root characteristics (H^+ net flux, root activity, root pH, rhizosphere pH) of winter wheat (* $p < 0.05$)

	Shoot weight	Root weight	H^+ net flux	Root activity	Root pH
Root weight	0.90*	—	0.60	0.58	0.52
Shoot weight	—	0.90*	0.21	0.29	0.23
H^+ net flux	0.21	0.60	—	0.86*	0.85*