Effects of high NH$_4^+$ on K$^+$ uptake, culm mechanical strength and grain filling in wheat

Lingan Kong*, Mingze Sun, Fahong Wang, Jia Liu, Bo Feng, Jisheng Si, Bin Zhang, Shengdong Li and Huawei Li

Crop Research Institute, Shandong Academy of Agricultural Sciences, Jinan, Shandong, China

Edited by:
Zuhua He, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, China

Reviewed by:
Yihua Zhou, Chinese Academy of Sciences, China
Dai-Yin Chao, Shanghai Institute of Plant Physiology and Ecology, China

*Correspondence:
Lingan Kong, Crop Research Institute, Shandong Academy of Agricultural Sciences, 202 Gongyebei Road, Jinan City 250100, China
e-mail: kongling-an@163.com

INTRODUCTION

Ammonium (NH$_4^+$) is an important source of inorganic N and can be used by plants as the sole N source. However, high levels of ambient NH$_4^+$ can be toxic to plant growth, resulting in many symptoms, including stunted root growth, yield reduction and leaf chlorosis (Britto and Kronzucker, 2002; Balkos et al., 2010; Li et al., 2010; Ariz et al., 2011; Chen et al., 2013). In rice (Oryza sativa L.), the anatomical traits of the culm are altered when high N is provided, leading to a reduction in culm mechanical strength and an increase in lodging scores (Yang et al., 2009). When larger quantities of urea (transformed to NH$_4^+$ through urease hydrolysis) are supplied, the efficiency of silicon in imparting rigidity in rice plants at low N doses is greatly reduced (Idris et al., 1975). In cereal crop production, lodging resulting from low mechanical strength severely damages the vascular bundles (Kashiwagi et al., 2008), thereby affecting the transport of water, nutrients and reserves contained in vegetative organs to the developing grain and decreasing grain yield and quality. Little information is currently available concerning the effects of NH$_4^+$ on culm mechanical strength and reserve transport in wheat. However, field observations and experience show that when wheat is grown at a high N rate, there appears to be an increased occurrence of lodging compared with growth at a moderate N rate, indicating that culm strength is low under high N levels. In Arabidopsis thaliana, when excessive N is supplied, the N concentration in the biomass increases significantly, whereas the N remobilization efficiency (NRE) decreases compared with moderate N application (Masclaux-Daubresse and Chardon, 2011). In a previous study, we found that application of excessive urea decreases the export of flag leaf-stored protein to the developing grains (Kong et al., 2012). Therefore, improving the NRE is a good strategy for achieving a high grain yield and crop quality under high N conditions.

The majority of studies investigating the toxicity of NH$_4^+$ at high concentrations have been associated with NH$_4^+$ assimilation and ion imbalances due to the decreased uptake of essential cations, such as K$^+$, Mg$^{2+}$ and Ca$^{2+}$ (Barker et al., 1967; Roosta and Schjoerring, 2008; ten Hoopen et al., 2010), and they are often associated with the availability of K$^+$ in particular (Yang et al., 2009). Because NH$_4^+$ can be transported through plant K$^+$ transporters and channels, NH$_4^+$ toxicity may be attributed to unregulated NH$_4^+$ uptake via these transporters, especially at low K$^+$ levels (ten Hoopen et al., 2010). It was recently proposed that NH$_4^+$ toxicity in NH$_4^+$-fed plants originates from NH$_3$ uptake by plants through one component of the low-affinity transport system (LATS) for NH$_4^+$ and from interference with K$^+$ transport through the second component (Ariz et al., 2011). In Arabidopsis, stimulation of the NH$_4^+$ efflux in the elongation zone following treatment with elevated NH$_4^+$ is linked to root growth inhibition by NH$_4^+$ (Li et al., 2010). The NH$_4^+$ efflux significantly enhances
futile and energy-costly NH$_4^+$ cycling at the plasma membrane in rice (Chen et al., 2013). Although a variety of hypotheses have been proposed to explain the mechanisms underlying NH$_4^+$ toxicity, no single convincing mechanism has yet been able to fully account for this toxicity (Roosta and Schjoerring, 2008; Chen et al., 2013).

Under field conditions, we have often observed the adverse effects of high NH$_4^+$ provision on wheat. Therefore, in this study, experiments were conducted in both the field and laboratory to examine the cellulose and lignin contents of the vascular bundle, culm mechanical strength and the NRE under treatment with moderate or high NH$_4^+$, or with high NH$_4^+$ combined with an elevated K$^+$ supply. The main objectives of this study were to investigate the effects of excessive external NH$_4^+$ on wheat growth and to determine whether elevated K$^+$ concentrations can alleviate these adverse effects of high NH$_4^+$.

**MATERIALS AND METHODS**

**PLANT MATERIALS**

A field experiment was conducted at an experimental station (36°42' N, 117°4' E; altitude 48 m) of the Shandong Academy of Agricultural Sciences, China. The climate in this region is continental and warm, with an average annual temperature of 13.6°C and an average rainfall of ~600 mm. The soil type was classified as sandy loam, with a pH of 7.2. The top 40 cm of the soil contained 2.13% organic matter, 66.2 mg kg$^{-1}$ hydrolysable nitrogen, 25.3 mg kg$^{-1}$ rapidly available phosphorous and 152.4 mg kg$^{-1}$ rapidly available potassium.

The winter wheat (Triticum aestivum L.) variety Jimai 22, developed by the Crop Research Institute of the Shandong Academy of Agricultural Sciences, Jinan, was used in the experiment and was sown on October 8, 2012, at a rate of 375 grains per m$^2$. The experiments were laid out in a split-plot design with three treatments and four replications. The treatments included moderate NH$_4^+$ (18 g N m$^{-2}$), high NH$_4^+$ (30 g N m$^{-2}$) and high NH$_4^+$ plus additional K$^+$ (K$_{add}$; 6 g K$_2$O m$^{-2}$). At sowing, 5 g N m$^{-2}$, 9 g P$_2$O$_5$ m$^{-2}$, and 9 g K$_2$O m$^{-2}$ were applied per treatment as basal nutrition. At the first node stage (the end of tillering), 13 g N m$^{-2}$, 25 g P$_2$O$_5$ m$^{-2}$, or 25 g N m$^{-2}$ plus 6 g K$_2$O m$^{-2}$ was top dressed in all three treatments, followed immediately by irrigation. N was supplied as NH$_4$Cl. The field-grown plants were used for data collection unless otherwise stated.

**MEASUREMENT OF CULM MECHANICAL STRENGTH**

The culm mechanical strength of the middle point of the basal second internode without a leaf sheath was measured using a handmade device. The device contains two semicircular semicircular grooves (5 cm apart) with approximately the same diameter as the wheat culm. During measurement, the second internode was set on the grooves, and a pallet was hung at the center of the internode; fine sand was then gradually added to the pallet until the stem broke. The total weight of the added sand and the pallet was subsequently determined using a balance. Culm mechanical strength was directly expressed as the weight required to break the internode.

**HISTOCHEMISTRY**

For histochemical localization of lignin, Wiesner reactions were performed using the method of Speer (1987). Briefly, transverse sections of the wheat culm second internode were cut freehand with a razor blade. Fresh sections were then incubated for 3 min in a 2% phloroglucinol (w/v), 95% EtOH solution, followed by 3 min of incubation in 50% HCl and subsequent mounting in 50% glycerol, in which phloroglucinol produces a red-pink product under acidic conditions, primarily through reaction with lignin cinnamaldehyde groups. The plant sections were examined directly under a light microscope, and digital images were recorded using an AxioCam MRC camera (Zeiss Axioskop 40, Leica, Germany). The optical density of stained lignin was quantified using the Image-Pro Plus 6.0 software (Media Cybernetics, Silver Springs, MD) and expressed on a scale of 0–2.

**FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY**

Freehand sections (c. 40 μm) were oven-dried at 60°C, and the vascular bundles were removed using a razor blade under an anatomical microscope before FTIR analysis. The FTIR spectra were recorded using an FTIR spectrometer (Magna-IR 750, Thermo Nicolet, Kanagawa, Japan) equipped with a Mercury–Cadmium–Telluride detector. The spectra of each sample were obtained in the range of 4000–400 cm$^{-1}$ at a resolution of 4 cm$^{-1}$ with 128 co-added interferograms and were normalized to obtain the relative absorbance.

**DETERMINATION OF SHOOT N CONTENTS**

Entire wheat shoots were dried at 70°C to constant weight and ground to pass through a 1-mm sieve. The samples (1 g of dry weight) were then placed in a Kjeldahl flask, and 20 ml of concentrated H$_2$SO$_4$ was added. After digestion, the solutions were cooled and diluted with deionized water to the specified volume. The total shoot N content was determined using the standard Kjeldahl procedure (Watkins et al., 1987). N remobilization is defined as the difference in the amount of shoot N between anthesis and the harvest stage. The NRE was calculated as the ratio between remobilized N and the total shoot N content at anthesis.

**GRAIN FILLING**

Based on grain development, five growth stages were designated for measurement: 0 days after anthesis (DAA) (end of anthesis); 8 DAA (milk development stage); 16 DAA (soft dough development stage); 24 DAA (hard dough development stage); and 32 DAA (ripening stage). The ears were collected at each stage and dried at 70°C for 48 h to a constant mass. The samples were then
MANUSCRIPT:

MEASUREMENT OF K⁺ CONTENTS
Fifteen germinated wheat seeds were transplanted into plastic basins (25 cm high and 20 cm in diameter) containing sterilized wet sand. The plants were then grown at 20–22°C under an 18-h photoperiod (white fluorescent light; 400 μmol m⁻² s⁻¹). The plants were watered daily with full-strength Hoagland’s nutrient solution (HNS) as a control; with HNS supplemented with 10 mM NH₄Cl as the high NH₄⁺ treatment; or with HNS supplemented with 10 mM NH₄Cl and 6 mM KCl as the high NH₄⁺ plus additional K⁺ treatment. For the K⁺ analysis, entire 40-day-old wheat plants were collected, washed with DD H₂O and oven-dried at 70°C for 48 h. The dried material was finely powdered and then subjected to wet digestion with HNO₃:HClO₄ (4:1) under shaking for 20 min at 200 rpm. The samples were filtered through Whatman No. 2 filter paper. The resulting solutions were appropriately diluted, and the K⁺ content was measured using a flame photometer (FP640, Shanghai, China).

K⁺ FLUX ANALYSIS USING THE SCANNING ION-SELECTIVE ELECTRODE TECHNIQUE (SIET)
For SIET, wheat seeds were surface sterilized in an aqueous solution of 1% NaClO for 5 min. The seeds were then washed several times with sterilized water, placed on wet filter paper in Petri dishes and incubated in distilled water. After germination, the seeds were transferred to larger plastic vessels and cultured hydroponically in full-strength HNS. The net K⁺ fluxes into the root epidermal cells were measured noninvasively in 15-day-old seedlings using SIET (BIO-001A SIET system; Younger USA Sci. & Tech. Corp., Amherst, MA, USA; Applicable Electronics Inc., Forestdale, MA, USA and ScienceWares Inc., East Falmouth, MA, USA). Recordings of steady-state K⁺ fluxes were performed as described by Sun et al. (2009). Prior to the measurements, the probes were calibrated in a solution (0.05 mM NH₄NO₃, 0.05 mM KCl, 0.1 mM CaCl₂, and 0.3 mM MES, pH 6.0) for 10 min. The steady fluxes were assayed in measuring solution (0.1 mM NH₄NO₃, 0.1 mM KCl, 0.1 mM CaCl₂, and 0.3 mM MES, pH 6.0) containing 10 mM NH₄Cl for approximately 10 min to verify that a steady-state condition was reached. Then, the transient K⁺ fluxes were measured in measuring solution not containing 10 mM NH₄Cl.

STATISTICAL ANALYSIS
All of the data were subjected to analysis of variance (ANOVA) using the Data Processing System (DPS) statistical software (v.14.10, Refine Information Tech. Co., Ltd., Hangzhou, Zhejiang, China) (Tang and Zhang, 2013). The data are presented as the mean ± standard deviation. The treatment means were compared using the least significant difference (LSD) test at P < 0.05.

RESULTS
DECREASE IN CULM MECHANICAL STRENGTH DUE TO HIGH NH₄⁺
Because lodging often occurred from the base of the plants, we determined the culm mechanical strength of the basal second internode of the wheat plants. Figure 1 shows that under field conditions, culm mechanical strength decreased continuously from anthesis to ripening. The application of high NH₄⁺ (30 g N m⁻²) led to significantly lower culm mechanical strengths, which were reduced by 29.20, 26.37, and 20.88% at 0, 15 and 30 DAA, respectively, with compared with the treatment with a moderate level of NH₄⁺ (18 g N m⁻²). Under high NH₄⁺ conditions, Kᵢadd improved culm mechanical strength by 26.95, 19.41, and 23.46% at 0, 15 and 30 DAA, respectively, with compared with the treatment without Kᵢadd.

A decrease in culm mechanical strength under high NH₄⁺ (HNS containing 10 mM NH₄⁺) (Figure S1B) and an improvement in the Kᵢadd treatment (Figure S1C) were also observed in wheat plants in a sand culture system. However, the adverse effect of high NH₄⁺ was only partially reversed by elevated K⁺.

DIFFERENTIAL EFFECTS OF HIGH NH₄⁺ AND Kᵢadd ON CELLULOSE AND LIGNIN CONTENTS
To determine whether high NH₄⁺ affects the contents of lignin and cellulose and whether elevated K⁺ modifies their biosynthesis and localization, culm cross-sections from the different treatments were stained with Calcofluor, to visualize cellulose, or with Wiesner reagents, to visualize lignin (Figures 2A, 3). As shown in the obtained images and through analysis with Image-pro Plus 6.0 software, the cellulose fluorescence intensity, particularly in the vascular bundles, was weaker in high-NH₄⁺-treated wheat (Figures 2B, E, G) than in moderate-NH₄⁺-treated wheat (Figures 2A, D, G). When the wheat plants were exposed to high NH₄⁺, Kᵢadd significantly promoted cellulose deposition in the vascular bundles (Figures 2C, F, G). Similarly, the amount of lignin decreased in the internodes of the wheat plants under high NH₄⁺, as indicated by the weaker red-pink color observed (Figures 3B, E, G) and the measurements of optical density performed using the Image-Pro Plus 6.0 software, compared with the moderate NH₄⁺ treatment (Figures 3A, D, G). Additionally, Kᵢadd
absorbance intensity due to a high level of NH$_4^+$, the cellulose and lignin contents decreased, whereas the positive

spectrum of K$_2$O $\alpha$ treated with moderate NH$_4^+$ is generated through digital subtraction of the spectra of wheat (Lichtfouse et al., 1998; Kaushik et al., 2010). A prominent peak at 1248 cm$^{-1}$ is ascribed to the aliphatic saturated C-H stretching vibration that takes place in polysaccharides, particularly cellulose and hemicelluloses (Lichtfouse et al., 1998; Kaushik et al., 2010). An indistinct band at 897 cm$^{-1}$ is indicative of lignin (Kaparaju and Felby, 2010).

To quantitatively distinguish the changes in the cellulose and lignin contents of the vascular bundles, difference spectra were generated through digital subtraction of the spectra of wheat plants treated with high levels of NH$_4^+$ from those of plants treated with moderate NH$_4^+$ or through digital subtraction of the spectra of K$_2$O treatment from those without additional K$^+$ under high NH$_4^+$. In the different spectra recorded, distinct peaks appeared at 2920, 2850, 1248, 1130, and 897 cm$^{-1}$. The decreased absorbance intensity due to a high level of NH$_4^+$ indicated that the cellulose and lignin contents decreased, whereas the positive values due to elevated K$^+$ treatment under high NH$_4^+$ conditions suggested that the cellulose and lignin contents increased in the vascular bundles of wheat.

**CHANGES IN SHOOT N CONCENTRATIONS**

As expected, the total shoot N concentration (%) in wheat during grain filling was consistently higher in the excessive compared with the insufficient NH$_4^+$ treatment, suggesting that high NH$_4^+$ promoted N uptake by the plants (Table 1). At 0 DAA, the greater total shoot N concentration caused by a high NH$_4^+$ supply was further increased by the K$^+$add treatment. The shoot N concentration gradually decreased during the grain-filling period in all three treatments. However, the shoot N concentration decreased more sharply under moderate NH$_4^+$ than at high NH$_4^+$; as a result, high NH$_4^+$ led to a lower NRE compared with the moderate NH$_4^+$ treatment. Under high NH$_4^+$ conditions, the shoot N concentration was greater from 0 to 16 DAA in the K$^+$add treatment than in the treatment without additional K$^+$, but the concentrations in these treatments were similar at maturity (32 DAA); consequently, the K$^+$add treatment resulted in a greater NRE. These data strongly indicate that the K$^+$add treatment might increase the N-use efficiency under high NH$_4^+$ conditions.

**GRAIN-FILLING RATE**

The grain dry mass decreased when the wheat plants were treated with high NH$_4^+$, especially during the early stages of grain filling.

---

**FIGURE 2** | Microscopy images of Calcofluor staining of cellulose in sections of the second internode of wheat plants at 16 DAA, showing that the maximum cellulose content was mainly present in the vascular bundles and sclerenchyma. The relative differences in the cellulose contents of wheat under moderate NH$_4^+$ (18 g N m$^{-2}$) (A), high NH$_4^+$ (30 g N m$^{-2}$) (B) and high NH$_4^+$ combined with K$_2$O (16 g K$_2$O m$^{-2}$) (C) are shown. (D), (E), and (F): higher magnification images of the areas highlighted in (A), (B), and (C), respectively. The presence of cellulose is indicated by luminous yellow coloration. The images represent at least 15 cross-sections from different plants for each treatment. (G): Effects of moderate NH$_4^+$, high NH$_4^+$ and high NH$_4^+$ combined with K$_2$O on cellulose deposition in the cell wall of the vascular bundles. The fluorescence intensity was quantified using Image-pro Plus 6.0 software. The columns labeled with different letters differed significantly at *P* < 0.05. ep, epidermis; pa, parenchyma cells; ph, phloem; sc, sclerenchyma cells; vb, vascular bundle; xy, xylem. Bars (A–C) 200 μm; (D–F) 800 μm.
the elevated K\(^+\) wheat plants that were not treated with 10 mM NH\(_4\)Cl, the whole wheat plants compared with the moderate NH\(_4\)Cl adding 10 mM NH\(_4\)Cl to the measuring solution resulted in a net K\(^+\) grain dry mass throughout the grain-filling period (in wheat seedlings, we measured the net K\(^+\) root epidermal cells using SIET (Figure 1)). As expected, under high NH\(_4\)Cl conditions, the K\(_{\text{add}}\) treatment increased the K\(^+\) content and improved culm mechanical strength (Figure 1). However, K\(_{\text{add}}\) only partly relieved the significant reduction in K\(^+\) contents caused by high NH\(_4\)Cl (Figure 5).

**INHIBITION OF K\(^+\) UPTAKE UNDER HIGH NH\(_4\)Cl**

A sand culture experiment was conducted to evaluate K\(^+\) uptake by the wheat plants using flame photometry. The high NH\(_4\)Cl treatment significantly decreased the K\(^+\) concentration in whole wheat plants compared with the moderate NH\(_4\)Cl supply (P < 0.01). As expected, under high NH\(_4\)Cl conditions, the K\(_{\text{add}}\) treatment increased the K\(^+\) content and improved culm mechanical strength (Figure 1). However, K\(_{\text{add}}\) only partly relieved the significant reduction in K\(^+\) contents caused by high NH\(_4\)Cl (Figure 5).

**ROOT K\(^+\) FLUXES IN RESPONSE TO HIGH NH\(_4\)Cl**

To further evaluate whether high NH\(_4\)Cl influences K\(^+\) uptake in wheat seedlings, we measured the net K\(^+\) flux responses in root epidermal cells using SIET (Figure 6A). In the roots of wheat plants that were not treated with 10 mM NH\(_4\)Cl, the net K\(^+\) influx into the root epidermal cells was determined (Figure 6B). SIET analyses showed that the average influx rate was approximately 17.88 pmol cm\(^{-2}\) s\(^{-1}\) (Figure 6C). However, adding 10 mM NH\(_4\)Cl to the measuring solution resulted in a remarkable decrease in the K\(^+\) influx rate, which dropped to an average of 2.75 pmol cm\(^{-2}\) s\(^{-1}\) (Figure 6C).

**DISCUSSION**

Previous studies have shown that high N application leads to thinner stems and significant increases in lodging, the lodging angle and the lodging score in wheat (Tripathi et al., 2003) and rice (Yang et al., 2009). Under a high N level (240 kg ha\(^{-1}\), supplied as urea), the culm and root strengths of wheat were found to be 20% and 17% weaker, respectively, compared with the application of 160 kg N ha\(^{-1}\) (Crook and Ennos, 1995), suggesting that high N application led to a lower culm strength and an increased occurrence of lodging. However, these investigations did not provide information explaining the mechanisms underlying these results. Because the basal part of the culm plays an important role in lodging resistance, as it provides a lever to hold the plant upright (Neenan and Spencer-Smith, 1975), and because culm breakage usually occurs at lower internodes (Kashiwagi et al., 2008), we determined the culm mechanical strength of the basal second internode. The results showed that the application of high NH\(_4\)Cl significantly decreased culm mechanical strength.

Nitrogen and potassium are two macroelements that are essential for plant growth. Although plants exhibit a wide variety of transport systems for the acquisition of these elements, competition for uptake between plants still exists and has become one of the main topics of studies conducted by biologists and...
agronomists. Rice growth is negatively affected by elevated NH$_4^+$, particularly under low K$^+$ levels, and NH$_4^+$ toxicity could be relieved by elevated K$^+$ (Balkos et al., 2010). Similar findings have been reported in Arabidopsis (Li et al., 2010). Therefore, we expected that elevated K$^+$ could compensate for the reduction in culm mechanical strength observed under high NH$_4^+$ conditions. Indeed, in this study, we found that K$_{add}^+$ alleviated the negative effects of high NH$_4^+$ on culm strength (Figure 1 and Figure S1). The data provided herein suggest that the plant K$^+$ status might be involved in the high NH$_4^+$-induced reduction in culm mechanical strength. These results are highly consistent with those of previous studies. In a field trial with maize (Zea mays L.), the application of K$^+$ was shown to significantly affect stem strength and stalk breakage (Melis and Farina, 1984). In rice, the K$^+$ culm content is closely correlated with culm mechanical strength because proper K$^+$ nutrition is associated with the lignification of sclerenchyma cells and vascular bundles, thereby strengthening the culms and increasing lodging resistance (De Datta and Mikkelsen, 1985; Zhang et al., 2010). To date, there is no direct evidence to support that K$^+$ strengthens cellulose and lignin deposition. However, it is widely accepted that K$^+$ play a key role in photosynthesis and metabolism of the resulting carbohydrates in plants (White and Karley, 2010; Hafsi et al., 2014). Considering that both cellulose and lignin are carbohydrates or its derivate and several pieces of indirect evidence presented herein are supportive, we could postulate that K$^+$ is involved in the cellulose and lignin deposition and thus in culm mechanical strength.

Mechanical strength is largely dependent on the chemical and biochemical components of the cell wall (Kashiwagi and Ishimaru, 2004; Kashiwagi et al., 2008). Generally, lignin and cellulose, which are the main biochemical components of plant tissues, particularly in the vascular bundles, are closely associated with culm mechanical strength (Yang et al., 2009). Cellulose usually constitutes 20–30% or 40–90% of the dry weight of
primary or secondary walls, respectively, varying with the cell type (Taylor et al., 1999). Moreover, lignin can be incorporated into the cell wall to enhance its mechanical strength. In the rice mutant brittle culm 1 (bcl), altered biosynthesis of cellulose, hemicellulose and lignin in the culms reduces secondary cell wall thickness and mechanical strength (Li et al., 2003). In wheat, the expression of COMT, a gene involved in lignin biosynthesis in the developing culm, is associated with culm rigidity and lodging traits (Ma et al., 2002). The TaCAD1 gene is also responsible for lignin synthesis, and the roles of lignin in maintaining stem strength and lodging resistance were further confirmed in maize (Halpin et al., 1998) and sorghum (Sorghum vulgaris Pers.) (Sattler et al., 2009) using CAD mutants. In the present study, through FTIR, histochemistry and Image-Pro Plus software analyses, we found that the application of high NH$_4^+$ decreased the cellulose and lignin contents in the vascular bundles of the second internode (Figures 2–4). In maize, K$^+$ stimulates rapid expression of phenylalanine ammonia lyase and enhances the activities of tyrosine ammonia lyase, cinnamyl alcohol dehydrogenase and phenoloxidase, thereby increasing lignin biosynthesis (Liu et al., 2007). Considering that culm strength is correlated with the contents of cellulose and lignin and, more importantly, with K$^+$ status, we speculate that an elevated K$^+$ supply likely alleviates the negative effect of NH$_4^+$ on the deposition of cellulose and lignin in vascular bundles. Indeed, using FTIR and histochemistry, we observed that the K$_{add}^+$ treatment increased the contents of both of these cell wall components (Figures 2–4).

It is widely known that as major components, cellulose and lignin are mainly deposited in the walls of certain specialized cells, such as the tracheary elements, sclerenchyma and phloem fibers (also shown in the present study; Figures 2, 3). Considering that cellulose and lignin impart rigidity and structural support to the wall and strongly assist in the transport of water and nutrients within xylem tissue by decreasing the permeability of the cell wall (Ma et al., 2002), we decided to investigate the transport of shoot reserves to developing organs. In this study, we found that high NH$_4^+$ nutrition influenced N transport from vegetative organs to the developing grains (Table 1) and decreased the grain-filling rate (Table 2). Given that carbohydrates account for the majority of the wheat grain composition and that more significant differences in the grain-filling rate than in the NRE are

### Table 2 | Effects of NH$_4^+$ and K$^+$ on grain dry mass (mg ear$^{-1}$) during grain filling.

| NH$_4^+$/K$^+$ rate (g m$^{-2}$) | Developmental stage (DAA$^a$) | 0 | 8 | 16 | 24 | 32 |
|-----------------------------|---------------------------|---|---|---|---|---|
| N$_m$ (18)                  | 0.28 ± 0.02 $^{a2}$      | 0.64 ± 0.02a | 1.06 ± 0.07a | 1.28 ± 0.06ab |
| N$_m$ (30)                  | 0.24 ± 0.02b             | 0.59 ± 0.02b | 1.03 ± 0.04a | 1.25 ± 0.03b |
| N$_m$ (30) +                | 0.27 ± 0.01ab            | 0.63 ± 0.03a | 1.11 ± 0.07a | 1.36 ± 0.07a |
| K$_{add}$ (g)               |                          |              |              |              |
| K$_2$O (kg$^{-1}$)          |                          |              |              |              |

$^a$DAA, days after anthesis.

$^b$The values represent the means and standard deviations of four replicates.

Means followed by the same letters within a column are not significantly different by Tukey’s test ($P < 0.05$).
observed between different treatments (Tables 1, 2), the decrease in the grain-filling rate may suggest that the nutrient translocation efficiency is not only determined by the lower permeability of the cell wall, but by the tissue C/N balance as well. Interestingly, elevated K+ also alleviates the negative effects of high NH₄⁺ on N remobilization and grain filling. These findings are consistent with previous studies in Arabidopsis thaliana in which the NRE was shown to be greater under low N than under a high N supply (Masclaux-Daubresse and Chardon, 2011), and the toxicity of high NH₄⁺ can be alleviated by K+ supplementation (Cao et al., 1993).

NH₄⁺ nutrition dramatically affects cation uptake by plants, leading to a reduction of cation contents (Szczerba et al., 2006, 2008). The mechanism underlying this effect is unknown, but it is commonly considered to result from direct competition between NH₄⁺ and other cations for the transmembrane through common pathways (ten Hoopen et al., 2010). In particular, K⁺ and NH₄⁺ may use the same channels because these cations are highly similar regarding their charge, size and hydration energy, which are characteristics that are important for membrane transport (Wang et al., 1996; White, 1996; Szczerba et al., 2008). K⁺ channels are an important component of the LAT5 for NH₄⁺ (ten Hoopen et al., 2010). Therefore, it has been speculated that these negative effects of high NH₄⁺ on wheat might be related to the modification of K⁺ flux in root cells.

In the present study, a high NH₄⁺ supply decreased the K⁺ content in wheat seedlings (Figure 5), indicating that high NH₄⁺ suppressed K⁺ uptake by the plants. Furthermore, we examined the net K⁺ flux across the root epidermal cells in SIET experiments and further confirmed that high NH₄⁺ decreased the K⁺ influx into the root epidermal cells (Figure 6). The results presented herein strongly suggest that uptake competition for NH₄⁺ over K⁺ mediates K⁺ transport under high NH₄⁺ conditions. This speculation is strongly supported by studies using intact barley seedlings, in which K⁺ fluxes into the root were shown to be much lower in seedlings grown using 10 mM NH₄⁺ compared with seedlings grown using 10 mM nitrate (NO₃⁻), and elevated K⁺ was able to ameliorate NH₄⁺ toxicity (Kronzucker et al., 2003; Szczerba et al., 2006). The authors proposed that this protection may be associated with the restoration of a moderate K⁺ status to the plant, a process that ultimately depends on K⁺ fluxes into the roots and its subsequent translocation to the shoots (Kronzucker et al., 2003; Szczerba et al., 2006). The suppression of the K⁺ influx at the plasma membrane may be due to the inhibitory action of high NH₄⁺ on high-affinity KUP/HAK/KT transporters (Spalding et al., 1999).

K⁺ and NH₄⁺ exhibit numerous similarities, including their size and charge, and are poorly distinguished by some channels and transporters (Wang et al., 1996; White, 1996; Szczerba et al., 2008; Ariz et al., 2011). One hypothesis to explain how K⁺ ameliorates the toxicity of NH₄⁺ is that K⁺ decreases the uptake of NH₄⁺. Indeed, suppression of the NH₄⁺ influx by K⁺ within minutes of increasing the K⁺ supply was observed in rice cultured in climate-controlled growth chambers under fluorescent lights (Balkos et al., 2010). Therefore, we examined this effect in wheat seedlings through SIET analyses and found that a high K⁺ rate did not alter NH₄⁺ flux but stimulated NO₃⁻ influx (data not shown) and thus high K⁺ treatment increased the total N uptake. This effect was also revealed by higher shoot N concentration in wheat plants under high K⁺ (Table 1). In field, plants grown under a slightly high NH₄⁺ or NO₃⁻ conditions show no toxicity on canopy growth but usually develop a weak culm mechanical strength as indicated by the higher lodging when strong wind occurs during grain filling. Given that the high K⁺ do not suppress the N uptake, the positive effects of K⁺ on cellulose and lignin deposition might be contributed to the additional K⁺ treatment in this study. Inhibition of K⁺ uptake by NH₄⁺ has also been observed in Arabidopsis by Cao et al. (1993); these authors reported that the protective effect of K⁺ was not due to inhibition of NH₄⁺ uptake. Additionally, as an intermediate of N metabolism and the most abundant amino acid in plants grown on NH₄⁺ -containing media (Hachiya et al., 2012), glutamine may be involved in the NH₄⁺ toxicity. Therefore, experiments are required to investigate the relationships among glutamine content and K⁺ uptake and there effects on cellulose and lignin deposition and culm mechanical strength under different levels of K⁺ supply and to elucidate the mechanisms of the morphogenesis of cellulose and lignin phenotypes.

In summary, based on the data provided herein, we conclude that at high external concentrations, NH₄⁺ decreases culm mechanical strength, cellulose and lignin contents, N remobilization from vegetative organs to the grain, the grain-filling rate and K⁺ uptake by wheat plants. These effects can be partially reversed by providing an additional K⁺ supply, most likely via competition with NH₄⁺ uptake and translocation. Thus, an understanding of the roles of interaction between NH₄⁺ and K⁺ in the regulation of culm mechanical strength and grain filling will be necessary to improve lodging resistance and productivity in wheat.

ACKNOWLEDGMENTS

This work was supported by the Shandong and National Earmarked Fund for Modern Agro-industry Technology Research Systems (SDAIT-04 and CARS-3-1-21) and the Special Fund for Agroscientific Research on Public Causes, MOA of China (201303109-7, 201203079, and 301203033-21).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/10.3389/fpls.2014.00703/abstract

Figure S1 | Forty-day-old sand-cultured wheat seedlings showing differences in culm mechanical strength under moderate NH₄⁺ (full-strength HNS; (A), high NH₄⁺ (full-strength HNS + 10 mM NH₄⁺, (B) and high NH₄⁺ with 6 mM additional K⁺ (C). The image represents five replicates.

REFERENCES

Ariz, L., Cruz, C., Moran, J. F., González-Moro, M. B., García-Olaverri, C., González-Murua, C., et al. (2011). Depletion of the heaviest stable N isotope is associated with NH₄⁺/NH₃ toxicity in NH₄⁺-fed plants. BMC Plant Biol. 11:83. doi: 10.1186/1471-2229-11-83
Balkos, K. D., Britto, D. T., and Kronzucker, H. J. (2010). Optimization of ammonium acquisition and metabolism by potassium in rice (Oryza sativa L. cv. IR-72). Plant Cell Environ. 33, 23–34. doi: 10.1111/j.1365-3040.2009.02046.x

Kong et al.
High NH₄⁺ inhibits K⁺ influx and N remobilization in wheat

Frontiers in Plant Science | Plant Physiology December 2014 | Volume 5 | Article 703 | 8
Li, Y., Qian, Q., Zhou, Y., Yan, M., Sun, L., Zhang, M., et al. (2003). Li, Q., Li, B. H., Kronzucker, H. J., and Shi, W. M. (2010). Root growth inhibition in Arabidopsis thaliana due to toxic and utilizable NH4+. J. Exp. Bot. 61, 2131–2142. doi: 10.1093/jxb/erq055

Melis, M., and Farina, M. P. W. (1984). Potassium effects on stalk strength, premature death and lodging of maize (Zea mays L.). South Afr. J. Plant Soil 1, 122–124. doi: 10.20527/sajps.1984.10634125

Neeman, M., and Spencer-Smith, J. L. (1975). An analysis of the process of lodging with particular reference to wheat and barley. J. Agric. Sci. 85, 495–507. doi: 10.1017/S0021859600062377

Roosta, H. R., and Schjoerring, J. K. (2008). Root carbon enrichment alleviates ammonium toxicity in cucumber plants. J. Plant Nutr. 31, 941–958. doi: 10.1080/0140184080243270

Sattler, S. E., Saathoff, A. I., Haas, E. I., Palmer, N. A., Funnell-Harris, D. L., Sarath, G., et al. (2009). A nonsense mutation in a cinnamyl alcohol dehydrogenase gene is responsible for the sorghum brown midrib phenotype. Plant Physiol. 150, 384–395. doi: 10.1104/pp.109.136408

Spalding, E. P., Hirsch, R. E., Lewis, D. R., Qi, Z., Sussman, M. R., and Lewis, B. D. (1999). Potassium uptake supporting plant growth in the absence of AKT1 channel activity. Inhibition by ammonium and stimulation by sodium. J. Gen. Physiol. 113, 909–918. doi: 10.1085/jgp.113.6.909

Speer, E. O. (1987). A method of retaining phloroglucinol of lignin. Stain Technol. 62, 279–280.

Sun, J., Chen, S., Dai, S., Wang, R., Li, N., Shen, X., et al. (2009). NaCl-induced alternations of cellular and tissue ion fluxes in roots of salt-resistant and salt-sensitive poplar species. Plant Physiol. 149, 1141–1153. doi: 10.1104/pp.108.129494

Szczerska, M. W., Britto, D. T., Balkos, K. D., and Kronzucker, H. J. (2008). Alleviation of rapid, futile ammonium cycling at the plasma membrane by potassium reveals K+-sensitive and -insensitive components of NH4+ transport. J. Exp. Bot. 59, 303–313. doi: 10.1093/jxb/erm309

Szczerba, M. W., Britto, D. T., and Kronzucker, H. J. (2006). Rapid, futile K+ cycling and pool-size dynamic define low-affinity potassium transport in barley. Plant Physiol. 141, 1494–1507. doi: 10.1104/pp.106.082701

Tang, Q. Y., and Zhang, C. X. (2013). Data Processing System (DPS) software with experimental design, statistical analysis and data mining developed for use in entomological research. Insect. Sci. 20, 254–260. doi: 10.1111/j.1744-7917.2012.01519.x

Taylor, N. G., Scheible, R. W., Cutler, S., Somerville, C. R., and Turner, S. R. (1999). The irregular xylem3 locus of Arabidopsis encodes a cellulose synthase required for secondary cell wall synthesis. Plant Cell 11, 769–780. doi: 10.1105/tpc.11.3.769

Ten Hoopen, E., Cain, T. A., Pedas, P., Hegelund, J. N., Shabala, S., Schjoerring, J. K., et al. (2010). Competition between uptake of ammonium and potassium in barley and Arabidopsis roots: molecular mechanisms and physiological consequences. J. Exp. Bot. 61, 2303–2315. doi: 10.1093/jxb/erq057

Tripathi, S. C., Sayre, K. D., Kaul, J. N., and Narang, R. S. (2003). Growth and morphology of spring wheat (Triticum aestivum) L culms and their association with lodging: effects of genotypes, N levels and ethephon. Field Crops Res. 84, 271–290. doi: 10.1016/S0378-4290(03)00095-9

Wang, M. Y., Siddiqui, M. Y., and Glass, A. D. M. (1996). Interactions between K+ and NH4+: effects on ion uptake by rice roots. Plant Cell Environ. 19, 1037–1046. doi: 10.1111/j.1365-3040.1996.tb0210x

Watkins, K. L., Vemal, T. L., and Krause, G. F. (1987). Total nitrogen determination of various samples types: a comparison of the Hach, Kjeltac, and Kjeldahl methods. J. Assoc. Off. Anal. Chem. 70, 410–412.

White, P. J. (1996). The permeation of ammonium through a voltage independent K+ channel in the plasma membrane of rice roots. J. Membr. Biol. 152, 89–99. doi: 10.1007/BF002329900088

White, P. J., and Karley, A. J. (2010). “Potassium,” in Plant Cell Monographs 17: Cell Biology of Metals and Nutrients, eds R. Hell and R. R. Mendel (Berlin; Heidelberg: Springer-Verlag), 199–224. doi: 10.1007/978-3-642-10613-2_9

Yang, S. M., Xie, L., Zheng, S. L., Li, J., and Yuan, J. C. (2009). Effects of Na rate and transplanting density on physical and chemical characteristics and lodging resistance of culms in hybrid rice. Acta Agron. Sin. 35, 93–103. doi: 10.3724/SP.J.1006.2009.00093

www.frontiersin.org

December 2014 | Volume 5 | Article 703 | 9
Zhang, F., Jin, Z., Ma, G., Shang, W., Liu, H., Xu, M., et al. (2010). Relationship between lodging resistance and chemical contents in culms and sheaths of japonica rice during grain filling. *Rice Sci.* 17, 311-318. doi: 10.1016/S1672-6308(09)60032-9

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 August 2014; accepted: 25 November 2014; published online: 16 December 2014.