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Mechanical properties of stem and physiological–biochemical responses of cucumber under different N and K conditions

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ORIGINAL ARTICLE

Abstract

In order to investigate the effects of N and K on the mechanical properties of cucumber stems, the physiological and biochemical indexes under different N and K conditions were compared. The analysis showed that the photosynthetic rate was affected by both stress and the self-senescence of cucumber plants, and that the crops under N and K stress were stimulated to activate a highly effective antioxidant system to cope with the damage. Sugar, as the basis for the synthesis and structure formation of the stems, was highly related to stem strength, and polyphenol oxidase in the study of stem strength was limited but indispensable. Compared with CK, the epidermal penetration strength under LN, LNLK, and LNHK treatments increased by at least 10%, while the strength under HN, LK, HNLK, and HNHK treatments decreased by at least 7%. To sum up, it was the photosynthesis rate, the sucrose transportation, as well as the futile cycling of K^+ that were the keys of stem strength.

Keywords: greenhouse cucumber; mechanical properties; nitrogen stress; physiological and biochemical indexes; potassium stress; stem

Introduction

Cucumber, an important vegetable crop with a wide cultivation area, is assuming great importance because its fruits are delicious with nutrient enrichment and fragrance, as well as often used in beauty products (Zhang *et al.*, 2020, 2021a). The total planting area in China exceeded half of the cucumber planting area worldwide (Cao *et al.*, 2021), which was second only to tomatoes in China (Wang *et al.*, 2020b). Hence, studying the response of cucumber to stress and improving the yield and quality are of great significance for the completion of the "vegetable basket" project and the maintenance of national food safety (Li *et al.*, 2021a; Wang *et al.*, 2020a).

The global population was expected to reach 9.6 billion by 2050, which required crop yields to increase by more than 85% compared to 2013 (Mu and Chen, 2021); an increasing amount of fertilizers was therefore used in modern agricultural crop production to meet the requirements of the rapidly escalating population for crop yield and quality (Waqas *et al.*, 2021; Zhu *et al.*, 2020). The amount of N fertilizers applied globally had increased 10-fold in the past 60 years (Huang *et al.*, 2021), and the K fertilizer requirements in China had far exceeded mined K resources (Li *et al.*, 2021d), which not only caused pollution, destruction of ecological balance and soil nutrients but also had a huge impact on human economic benefits and health (Chen *et al.*, 2018; Sabzi *et al.*, 2021). Therefore, investigating the response of crops to NK and balancing fertilizer application are the bases of the need to balance benefits and costs and avoid environmental degradation (Huang *et al.*, 2021; Li *et al.*, 2021c; Zhu *et al.*, 2020).

N and K are the basic elements of crop growth and are essential for enhancing the quantity and quality of the harvested produce (Li *et al.*, 2021b, 2021d). N affected crop growth externally by reducing leaf area and green leaf lifespan, and internally by inhibiting the synthesis of chlorophyll and protein to reduce photosynthetic products (Dos Santos *et al.*, 2020; Mu and Chen, 2021). K^+ participated in protein synthesis, photosynthesis, enzyme activation, regulation of osmosis, and ion homeostasis (Li *et al.*, 2021c), and the research of Yang, H. *et al.* showed that under low K^+ condition, the K^+ concentration of old leaves decreased, but the concentration of new leaves changes little because of the highly mobile potassium in the crop (Yang *et al.*, 2020). In addition, phloem transportation and biomass partitioning also depended on the regulation of K^+ (Li *et al.*, 2021d). As a signal, reactive oxygen species (ROS) played an important role in the physiological process of crop response to stress (Wang *et al.*, 2013), but excessive ROS induced oxidative damage of the plasma membrane and produced malondialdehyde (MDA), which was also one of the main factors of reduced photosynthesis (Pi *et al.*, 2014). In order to cope with the damage of ROS, in the initial stage of stress, crops defended by increasing the activity of catalase (CAT) and superoxide dismutase (SOD) (Yang *et al.*, 2021). The sugars in tomato (Sung *et al.*, 2015), rice (Khan *et al.*, 2021), stevia (Sun *et al.*, 2021), and corn (Ma *et al.*, 2020), as the main form of photosynthetic products, all had significant changes under stress. Polyphenol oxidase (PPO), which is widely present in animals and plants, was a key enzyme determining the color of mammalian skin, hair, fruits and vegetables, and food softening (Kim *et al.*, 2005; Ren *et al.*, 2021). It was reported by Kunito, T., *et al.* that PPO promoted the degradation of cellulose and lignin in the soil, and its activity decreased with the increase of N (Kunito *et al.*, 2009), which was of importance for the study of stem strength.

There was a tight relationship between NK and stem strength. Based on previous research, it could be concluded that low K and high N reduced the stem strength (Li *et al.*, 2021d; Xue *et al.*, 2017) mainly by inhibiting the synthesis of cellulose and lignin or changing the structure of the cell wall. The production and partitioning of biomass were determined by N and K, and subsequently, the composition content of the stem and the structure of the cell wall were affected. Furthermore, the stems were easier to be located and observed because they were not covered compared with the roots and had a smaller number compared with the leaves. However, the current research on stem strength was mainly used to investigate the lodging resistance characteristics of crops (Li *et al.*, 2021d; Manga-Robles *et al.*, 2021; Sekhon *et al.*, 2020; Shao *et al.*, 2021; Xue *et al.*, 2017), which was extremely surprising and regrettable. Hence, as the only channel for nutrient transport and carbohydrate partitioning between roots and leaves, stems deserve more attention in crop response to stress. This study investigated the response of cucumber stem strength to N and K with the comparison of physiological and biochemical characteristics under different N and K conditions. These investigations are vital, not only for broadening application range of stem strength but also for understanding the response of crops to adversity with the mechanical properties of stems.

Materials and Methods

Test location and details

The “Jinyou 1” cucumber, which was acquired from Tianjin Academy of Agricultural Sciences, Tianjin, China, was used in the experiment in the Venlo greenhouse of Jiangsu University from June to July 2021. The seeds germinated and grew in the plug tray until true leaves appeared and expanded, and then were transplanted into a pot (diameter 29.5 cm, height 19.3 cm) filled with 8 L perlite. The seedlings were irrigated with Kawasaki solution (Jiheng *et al.*, 2011) once every morning from 8:00 to 9:00 a.m., 600 mL each time. The standard composition was: $Ca(NO_3)_2{\cdot}4H_2O$, 826 mg/L; KNO_3, 607 mg/L; NH_4H_2PO4, 114 mg/L; $MgSO_4{\cdot}7H_2O$, 429 mg/L; Fe-EDTA, 7 mg/L; $MnSO_4{\cdot}4H_2O$, 1.7 mg/L; $Na_2B_4O_7{\cdot}10H_2O$, 2.45 mg/L; $ZnSO_4{\cdot}4H_2O$, 1.45 mg/L; $CuSO_4{\cdot}5H_2O$, 0.19 mg/L; and $Na_2MoO_4{\cdot}2H_2O$, 0.12 mg/L. Finally, data collection was performed during the flowering period.

Experiment design

On the 10th day after transplanting, the seedlings were distributed within nine groups, after which nutrient treatments were initiated. There were three levels of nitrogen (50, 100, and 150%) and three levels of potassium (50, 100, and 150%) in the experiment, namely, CK (control group, 100%N+100%K), LN (50%N+100%K), HN (150%N+100%K), LK (100%N+50%K), HK (100%N+150%K), LNLK (50%N+50%K), LNHK (50%N+150%K), HNLK (150%N+50%K), and HNHK (150%N+150%K). Nutrition treatments were as follows: in the low-level treatment, $Ca(NO_3)_2$ and KNO_3 were replaced by $CaCl_2$ and KCl to halve the N and K elements, while in the high-level treatment, $NaNO_3$ and KCl were used to increase the N and K elements. In addition, because KNO_3 contained both N and K, KCl and $NaNO_3$ were used to supplement the K which was reduced in the low nitrogen treatment group and the N which was reduced in the low potassium treatment group, respectively. The specific cultivation plan is shown in Figure 1.

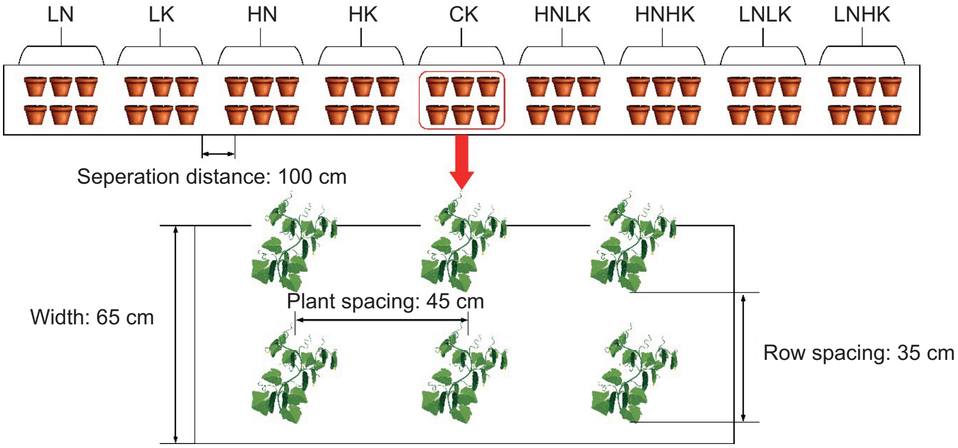

Figure 1. Diagram of experiment design.

Gas exchange parameters

Gas exchange parameters mainly included photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr). The data collection was done from 9:00 to 11:30 in the morning, because the water, fertilizer supply, and photosynthesis were stable enough to make the results reliable and accurately reflect the growth status of greenhouse cucumbers. Li-6400 (LI-COR Inc., Lincoln, NE, USA) was used to determine the parameters of the well-growing leaves, whose positions were similar under different treatments and were marked for follow-up testing. The parameters were measured before grouping treatment (10th day after transplanting), 1 week after treatment (17th day after transplanting), and before puncture test (24th day after transplanting). During the measurement, five plants were selected for each treatment, and stable data were recorded five times for each leaf.

Physiological and biochemical indexes

On the 24th day after transplanting, the hydrogen peroxide (H_2O_2) and enzyme activities in cucumber leaves and stems were measured after finishing the gas exchange parameters. The samples of leaves and stems were collected and immediately frozen in liquid nitrogen to ensure the activity of H_2O_2 and enzymes. The sample was ground and centrifuged at a low temperature, and then the absorbance of the reaction mixture in the supernatant at a specific wavelength was read by spectrophotometry (made by Yoke Instrument, Shanghai, China). H_2O_2 and CAT were calculated with the absorption peak of the peroxymolybdic acid complex, which was formed by the rapid combination of hydrogen peroxide and molybdate under acidic medium (Chai *et al.*, 2004). The extraction and determination of MDA followed the thiobarbituric acid reaction described by Pi, z. *et al.* (Pi *et al.*, 2014). The PPO activity was calculated with linear increase in absorbance of the product catalyzed (Ren *et al.*, 2021). Xanthine oxidase was used as one of the most convenient methods to determine the content of SOD because of the specific inhibitory effect of SOD on superoxide radical (Peskin and Winterbourn, 2017; Zhanshen, 2001).

The content of soluble sugar was calculated by the absorbance at 620 nm of the blue-green complex formed under the action of concentrated sulfuric acid (Xiaoxu and Jiazheng, 2013). The sucrose content was determined on the basis of the absorbance of colored substances formed by the reaction of resorcinol and fructose produced by hydrolysis (Shiyuan, 1998).

Stem strength

Stem strength can be predicted by measuring the force required to pierce the epidermis (Manga-Robles *et al.*, 2021). TA-XTPLUS texture analyzer (made by Stable Micro System, London, UK) was used to measure the epidermal penetration strength of cucumber stems in greenhouses. Five plants were selected for each treatment, and each plant was repeatedly punctured three times. The probe was moved from the surface of the stem to a depth of 10 mm at a speed of 2 mm·s^{-1} and a minimum sensitivity of 5 g.

Statistical analysis

Each treatment was repeated at least three times, and the mean values and standard deviations of each of the parameters studied, including gas exchange parameters, physiological and biochemical indexes, as well as Stem Strength were calculated. Analysis of variance (ANOVA) was performed using SPSS 18.0, and the least significant difference test (LSD) was used to determine significance at the level of $P < 0.05$.

Results

Gas exchange parameters

The gas exchange parameters of greenhouse cucumber leaves are shown in Figure 2. It can be seen from Figure 2 that Pn, Gs, and Tr of the leaves gradually decrease with cucumber growth. The Pn under the LN, LK, LNLK, and LNHK treatments was most severely affected on the 17th day compared with that on the 10th day, with a decrease of 34.97, 31.79, 45.93, and 24.90%, respectively, which far exceeded the 8.02% of the CK treatment. On the 24th day, the Pn under LN, LK, and LNLK treatments was only half of the initial state. With the extension of the treatment time, the Gs of each group decreased to a great extent. Exceptionally, the Gs under HK treatment on the 17th day was reduced by 5.49% compared with the initial state, which was much lower than the decrease of other treatments. On the 17th day, the Tr under LN, LK, and LNLK treatments decreased most obviously compared with that on the 10th day, while the decrease of Tr under CK, HK, LNHK, and HNHK treatments was the lowest. The Ci under CK treatment gradually decreased, while the other groups showed a tendency to first decrease and then increase. In particular, the Ci under LNLK treatment continued to increase.

As shown in Figure 2A, on the 17th day after transplanting, the Pn under LN, HN, LK, HK, LNLK, LNHK, HNLK, and HNHK treatments decreased by 27.31, 12.18, 24.17, 5.20, 39.34, 16.24, 7.96, and 9.10%, compared with that under the CK, and the Gs only increases under the HK treatment. On the 24th day, the Pn under LN, LK,

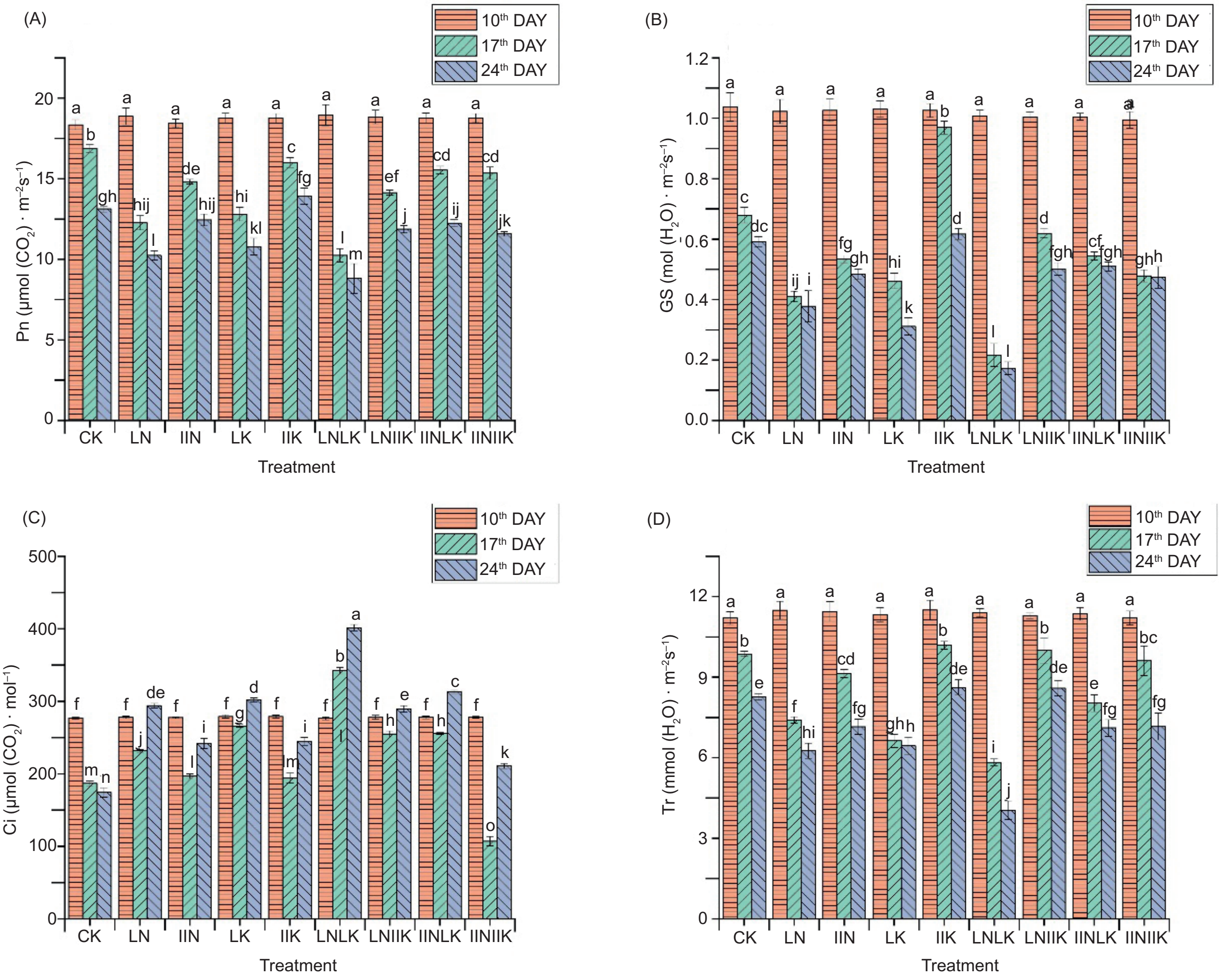

Figure 2. Effects of different water and fertilizer treatments on greenhouse cucumber gas exchange parameters. (A) Photosynthetic rate (Pn); (B) stomatal conductance (Gs); (C) intercellular CO_2 concentration (Ci); (D) transpiration rate (Tr).

LNLK, LNHK, HNLK, and HNHK treatments decreased by 21.99, 17.77, 9.56, 6.74, and 11.80%, respectively, compared with that on the 17th day. In comparison with the CK treatment, the Gs under the LN, LK, and LNLK treatments decreased by more than 35%, while that changed little under the HK treatment. High K$^+$ concentration was conducive to the bottom-up water transportation of cucumber; in contrast, continuous high K$^+$ stress or other treatments had significant toxic effects on transpiration, especially LNLK treatment. Except for the HNHK treatment on the 17th day, the Ci of the other treatments was higher than that of CK.

H$_2$O$_2$ and enzyme activity

The effect of treatment on greenhouse cucumber H$_2$O$_2$ and enzyme activity is shown in Table 1. It can be seen from Table 1 that compared with the CK treatment, NK stress and combined stress caused a H$_2$O$_2$ burst in cucumber stems and leaves, and the subsequent MDA accumulated rapidly, the amount accumulated of which was often used to indicate the degree of cell membrane damage when the lipid peroxidation caused by the continuous accumulation of H$_2$O$_2$ exceeded the tolerance (Helena Ramirez-Solet *et al.*, 2021; Zhang *et al.*, 2021b). Under the conditions of LN, LK, LNLK, and LNHK, the H$_2$O$_2$ and MDA in leaves and stems were the highest, while there was no significant effect of HK treatment on MDA in leaves and stems. The contents of H$_2$O$_2$ and MDA in the absence of N and K were significantly higher than the contents of excessive N and K, and the influence of N deficiency was dominant, which was also the case under combined stress. The H$_2$O$_2$ in the stem remained at a relatively low level in contrast to a large amount of accumulation in leaves, but the content of MDA in leaves and stems was similar.

As antioxidant enzymes, CAT and SOD are important means for crops to remove ROS and reduce oxidative damage (An *et al.*, 2020). Compared with CK treatment, HN, LK, and HK treatments induced an increase in CAT and SOD activity in leaves, while the activity decreased under LN, LNLK, LNHK, and HNLK treatments. In addition, the activity of CAT in the leaves under HNHK treatment hardly decreased; in contrast, the activity of SOD increased significantly. In the stem, the CAT and SOD activity under the treatments of LN, LNLK, and LNHK significantly reduced, while the effects of HN, LK, HK, and HNHK treatments on the activity of CAT and SOD were positive. It is worth noting that the changes of antioxidant enzymes in stems under HNLK treatment are similar to those of leaves under HNHK treatment, which seems to indicate that CAT and SOD are not completely consistent in the reaction logic. In addition, the antioxidant response of leaves and stems is also significantly

Table 1. Effects of different treatments on H$_2$O$_2$ and antioxidant enzyme activities in cucumber.

	Treatment	H$_2$O$_2$ (mmol·gprot^{-1})	MDA (nmol·mgprot^{-1})	CAT (U·mgprot^{-1})	SOD (U·g^{-1} FW)
Leaf	CK	0.426 ± 0.101^{g}	0.302 ± 0.041^{g}	7.659 ± 0.162^{d}	462.813 ± 23.768^{d}
	LN	2.757 ± 0.097^{b}	0.972 ± 0.046^{c}	1.613 ± 0.379^{g}	265.952 ± 12.164^{f}
	HN	1.261 ± 0.030^{e}	0.486 ± 0.044^{e,f}	11.145 ± 0.341^{b}	643.190 ± 19.620^{a}
	LK	2.177 ± 0.087^{c}	0.753 ± 0.039^{d}	9.877 ± 0.166^{c}	540.456 ± 6.084^{c}
	HK	0.937 ± 0.117^{f}	0.399 ± 0.041^{f,g}	14.585 ± 0.219^{a}	602.028 ± 11.420^{b}
	LNLK	3.925 ± 0.094^{a}	1.895 ± 0.129^{a}	1.206 ± 0.218^{g}	181.738 ± 18.799^{g}
	LNHK	2.915 ± 0.084^{b}	1.412 ± 0.150^{b}	3.821 ± 0.103^{f}	386.194 ± 21.658^{e}
	HNLK	1.858 ± 0.105^{d}	0.596 ± 0.038^{e}	6.217 ± 0.160^{e}	419.230 ± 9.664^{e}
	HNHK	2.040 ± 0.069^{c}	0.522 ± 0.042^{e,f}	7.354 ± 0.173^{d}	574.085 ± 16.119^{b,c}
Stem	CK	0.025 ± 0.011^{g}	0.218 ± 0.018^{g}	1.742 ± 0.078^{e}	312.349 ± 9.290^{e}
	LN	0.544 ± 0.016^{b}	0.977 ± 0.021^{b}	0.830 ± 0.093^{g}	152.088 ± 5.046^{g}
	HN	0.164 ± 0.018^{e}	0.331 ± 0.024^{f}	7.184 ± 0.378^{b}	594.326 ± 13.675^{a}
	LK	0.298 ± 0.011^{c}	0.690 ± 0.057^{d}	4.067 ± 0.351^{c}	505.203 ± 11.885^{c}
	HK	0.135 ± 0.010^{e,f}	0.250 ± 0.022^{f,g}	8.744 ± 0.086^{a}	542.954 ± 8.646^{b}
	LNLK	0.685 ± 0.030^{a}	1.246 ± 0.087^{a}	0.377 ± 0.172^{h}	116.480 ± 8.142^{h}
	LNHK	0.324 ± 0.008^{c}	0.825 ± 0.036^{c}	1.213 ± 0.085^{f}	269.878 ± 25.031^{f}
	HNLK	0.111 ± 0.016^{f}	0.531 ± 0.102^{e}	1.556 ± 0.096^{e,f}	395.783 ± 11.994^{d}
	HNHK	0.245 ± 0.011^{d}	0.342 ± 0.023^{f}	2.132 ± 0.090^{d}	416.937 ± 4.498^{d}

Different superscript lowercase letters indicate a statistically significant differences in the physiological and biochemical parameters of cucumbers under different treatments at *P* = 0.05.3.3. PPO different, as the antioxidant enzyme activity in the stems is significantly lower than that of the leaves, and the changes of CAT under HNHK treatment and SOD under HNLK treatment in the stem were also opposite to those in leaves.

The changes of PPO activity in stems and leaves are shown in Figure 3. PPO is very common in animals and plants (Ren *et al.*, 2021). As can be seen from Figure 3, the PPO activity under LN, HK, LNLK, and LNHK treatments increased significantly in contrast to the significant decrease under HN, LK, HNLK, and HNHK treatments. It is a remarkable fact that under the CK treatment and the treatment with an increase in activity, the PPO activity in the stem is higher than that in the leaves, while the PPO activity in the stem is lower under the treatment with decreased activity.

Sugar

The changes of soluble sugar and sucrose in stems and leaves are shown in Figure 4. It can be seen from Figure 4A that treatments have different effects on soluble sugar in comparison with CK treatment. The effect of LN treatment on the accumulation of soluble sugar is positive, and the effect of HN treatment is the opposite. Under LK treatment, the soluble sugar in leaves increased by 79.19%, while the soluble sugar in stems was decreased by 67.56%. Under HK treatment, the soluble sugar in the leaves was reduced by 12.58% in contrast to little change in the stem. As shown in Figure 4B, under LN, LK, LNLK, and HNLK treatments, the sucrose content in the leaves increased compared with CK treatment, while the content under HN, HK, LNHK, and HNHK decreased. For sucrose accumulation in the stem, the positive effects of LN, LNLK, LNHK, and HNHK treatments and the opposite effects of HN, LK, and HNLK treatments were both found (Figure 4).

Epidermal penetration strength

The change of epidermal penetration strength of cucumber stem is shown in Figure 5. The epidermal penetration strength under LN, LNLK, and LNHK treatments increased by 17.77, 10.08, and 31.96%, respectively, while the strength under HN, LK, HNLK, and HNHK treatments decreased by 17.64, 7.69, 12.01, and 24.34%, respectively. Exceptionally, the effect of HK treatment on the epidermal penetration strength of stem was not obvious. Manifestly, under single factor stress or combined stress, N played a much more essential role in the

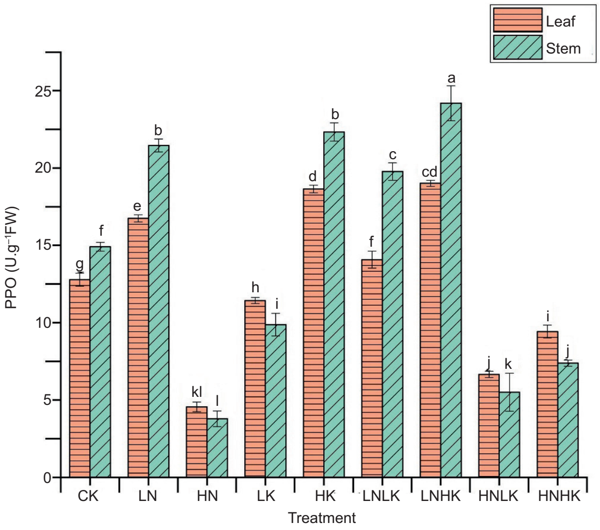

Figure 3. PPO activity in stem and leaf.

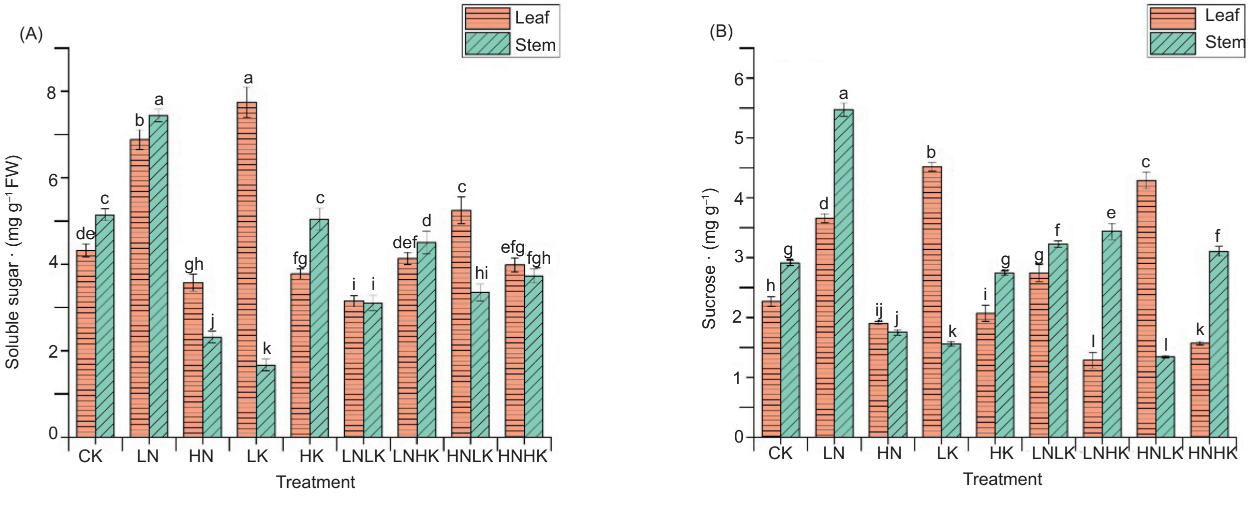

Figure 4. Sugar content in stem and leaf. (A) Soluble sugar; (B) sucrose.

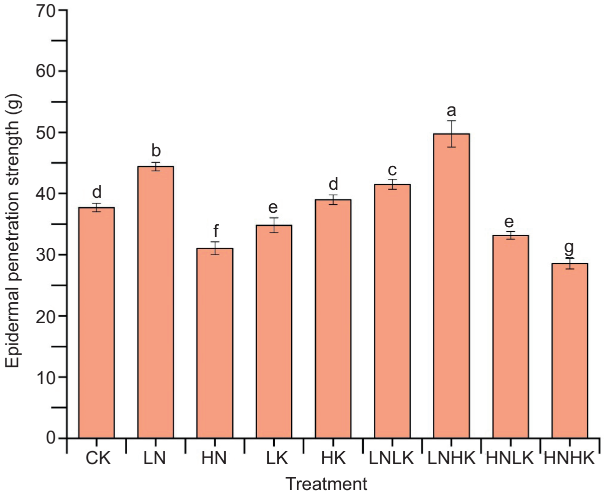

Figure 5. Epidermal penetration strength of stem.

mechanical properties of stem epidermis in comparison with K, which was mainly achieved by influencing structure construction and component synthesis.

Discussion

Combined stress is not a simple addition of independent stress effects, and the response of crops to K deficiency is usually masked by the symptoms of N deficiency, because the demand for K is also reduced during N deficiency (Zhu *et al.*, 2020, 2021). The continuous decrease of the Pn under the treatments of LN, LK, LNLK, and LNHK indicated that the effect of N deficiency on crops was more significant, and the N stress response was dominant in the combined stress (Zhu *et al.*, 2020). On the 17th day, the HK, LNHK, and HNHK treatments had almost no effect on the Tr in comparison with that on the 10th day, which was mainly because crops absorbed more water to avoid excessive penetration to a certain extent, thereby adapting to abnormal K supply through K^+ transport and transpiration (Xu *et al.*, 2018). The decrease in the assimilation rate of CO_2 induced by changes in water and fertilizer conditions promoted the accumulation of CO_2, while insufficient K supply interfered with the opening of the stomata, thereby exacerbating this accumulation (Boussadia *et al.*, 2010; Helena Ramirez-Solet *et al.*, 2021; Liu *et al.*, 2021). As a result, the Ci first decreased and then increased, except for the gradual decrease of CK treatment and the continuous increase of LNLK treatment. Gradual decrease of Pn, Gs, and Tr with the increase of treatment days, in which there was also a decline under the CK treatment, indicated that leaf senescence had a significant effect on gas exchange parameters, and this effect continued to increase with cucumber growth (Mu and Chen, 2021).

The hyperaccumulation of ROS under the conditions of LN, LK, LNLK, and LNHK destroyed the pH homeostasis in cell (Waqas *et al.*, 2021), which showed a continuous decline in photosynthesis on cucumber leaves. LNLK treatment caused serious damage to crops, because N and K deficiency not only made the crop lose the key elements for synthesizing protein and chlorophyll but also destroyed the homeostasis of the cell and increased the resistance to biomass transportation, and the damage further aggravated by the combination of N and K was even worse (Helena Ramirez-Solet *et al.*, 2021; Okazaki *et al.*, 2020; Zhang *et al.*, 2021b). The low MDA under HK treatment suggested that the crop alleviated the damage of high K stress through K^+ transport and transpiration, which in turn provides a stable physiological environment for the protection mechanisms (Xu *et al.*, 2018). The accumulation of H_2O_2 and the activity of antioxidant enzyme in the leaves were significantly higher than that in the stem, while the MDA in the leaves and the stem was almost at a similar level. It provided an insight into the adaptive response that H_2O_2, a signal molecule that regulates stomata, was more likely to accumulate in leaves (Waqas *et al.*, 2021) and stems were more sensitive to ROS damage and had weaker antioxidant capacity in comparison with leaves. With the accumulation of ROS, the antioxidant system responded naturally, causing an increase in the activity of CAT and SOD (Yang *et al.*, 2021). The decreased activity of CAT and SOD in leaves and stems under N deficiency or combined stress was attributable to the continuous accumulation of ROS that exceeded the tolerance, which destroyed the antioxidant response system of the cell (Pi *et al.*, 2014). In addition, the effect of NK supply on enzyme synthesis, which led to the suppression of the upper limit of the ability to remove ROS, was also a notable cause (Waqas *et al.*, 2021).

The soluble sugars in the stems and leaves under the LN treatment continued to accumulate because the crops increased outward transportation (Gao *et al.*, 2015), while under the HN treatment, the crops were more likely to leave photosynthetic products in the source organs, which illuminated the effect of HN treatment on leaf expansion (Hou *et al.*, 2021). K deficiency repressed carbohydrate metabolism and transport genes, and impaired the transport of photosynthetic products through the phloem to the sink organs, resulting in the continuous accumulation of sugar in the leaves and the loss of the source of photosynthesis products in the stems (Sun *et al.*, 2021) (Figure 3A). The reduction of soluble sugars under HK treatment may be related to the energy consumption of ion transport that alleviates osmotic stress (Xu *et al.*, 2018). The diversity of ingredients partially revealed the complexity of changes in soluble sugar under combined stress (Hu *et al.*, 2017; Sun *et al.*, 2021), and the effect of the combination of NK stress on the homeostasis of crops was another possible reason (Waqas *et al.*, 2021; Zhang *et al.*, 2021b).

Sucrose is the main form of transport of photosynthetic products (Cui *et al.*, 2020). Crops under stress adjusted the distribution of photosynthetic products among various organs to cope with sudden alterations in the external environment, and the process was always in a direction that was more conducive for survival (Boussadia *et al.*, 2010; Gao *et al.*, 2015; Yang *et al.*, 2021). Under the condition of N deficiency, the response that crops distributed more biomass to the root system to promote the growth of the root system was conducive to obtaining more nitrogen (Gao *et al.*, 2015), and the accumulation of the photosynthetic product, sucrose, therefore rose in the leaves and stems of crops (Figure 3B). However, the accumulation of sucrose under LNLK and LNHK treatments showed high similarity with symptoms under N deficiency, but the continued increase was not observed due to the limited stress resistance of sucrose transport. The lower sucrose in the leaves under LNHK treatment may be due to the removal of the restriction of K^+ on sucrose transport and the increased energy consumption of the osmotic regulation mechanism of high K^+ (Xu *et al.*, 2018). In addition, the sucrose accumulation was also limited by their destruction of photosynthesis (Waqas *et al.*, 2021). The process by which K^+ was absorbed by the root system into the xylem and transported to the leaves through transpiration, and then transported to the various organs through the phloem, seemed to be a futile cycle; on the contrary, it was complicated and crucial (Dreyer *et al.*, 2017). Related studies showed that K^+ served as a signal of potassium demand (Dreyer *et al.*, 2017) and was hardly metabolized in crop cells (Han *et al.*, 2016), which was the basis of the interesting cycle. In the futile cycle, K^+ actively participated in regulating the osmotic environment in crops, and the activities of carbohydrate metabolizing enzymes and transport enzymes (Sun *et al.*, 2021; Zhang *et al.*, 2021b). The restricted movement of sucrose to the sink organs under LK treatment resulted in the rise of sucrose in the leaves and the decline in the stems (Sun *et al.*, 2021) (Figure 3B).

PPO has always been the focus of research on the browning of fruits and vegetables (Ren *et al.*, 2021). Kunito, T. *et al.* found that PPO can promote the degradation of lignin in the soil, and the activity of PPO decreases after adding N (Kunito *et al.*, 2009). Similar effects may also be observed in plants. The result of the increase of PPO activity under N deficiency revealed the regulation of N. In contrast, the effect of LK treatment on PPO was negative, but this result still provided valuable evidence for the hypothesis that K+ played a role in PPO regulation to a certain extent. However, the weak negative correlation strongly suggested that the effect of PPO activity on the stem epidermal strength was limited and nondeterministic. Nevertheless, PPO should not be ignored in the study of stem mechanical properties, considering that PPO was indeed regulated by N and K, as well as contributed to the degradation of lignin, which was the mechanical basis of the stem, and the change of the stem structure through the combination with internal substances.

More research has shown that the stem strength is mainly determined by cellulose and lignin (Mahlanza *et al.*, 2014; Xue *et al.*, 2017). Under LN and HK treatments, sucrose was the main form of transport of photosynthetic products (Cui *et al.*, 2020), and its outward transport was enhanced (Gao *et al.*, 2015; Sun *et al.*, 2021), which was conducive for the synthesis of lignin and cellulose in the stems. In addition, the high K^+ concentration itself also promoted the lignification of the stem (Li *et al.*, 2021d). Therefore, the cucumber stem strength under the LN and HK treatments rose. However, it was because of the alleviating effect of K^+ transport on osmotic pressure that the change of stem strength under HK treatment was gentler than that of LN treatment (Xu *et al.*, 2018). The stem strength was significantly reduced under the HN and LK treatments on account of less photosynthetic products being transported to the stem. The reason for the decrease under LK treatment was that the transport of photosynthetic products to the phloem of the sink organs was restricted (Sun *et al.*, 2021); in contrast, the reason under the HN treatment was that the leaves retained more photosynthetic products. In addition, the weaker connection between adjacent cells and the instability of cell structure was also induced by low K^+ concentrations (Li *et al.*, 2010). However, upon the perception of K^+ deficiency, the mobilization mechanism of K^+ was activated, which improved the absorption and utilization efficiency of K^+ to a certain extent (Yang *et al.*, 2020). The complex mechanism delayed the onset of K deficiency symptoms, resulting in a significant lag in the reduction of stem strength under LK treatment in comparison with HN treatment. Although the complexity of combined stress is not explained by the independent addition of individual stress effects, it is very clear that the impact of combined stress is stronger than individual deficiency (Zhu *et al.*, 2020).

Conclusions

The analysis of physiological and biochemical indexes of greenhouse cucumber allowed us to shed light on the complex relationship between stem strength and NK. Under the individual or combined stress of N and K, the effect of nutrient deficiency on crops was more visible, and N stress response had priority in combined stress. In addition, the effect of combined stress was stronger than that of single stress. Leaf senescence had a significant effect on gas exchange parameters, and this effect continued to increase as growth progresses. The analysis of H_2O_2 and enzyme activity showed that the stimulated crop cells increased the activity of CAT and SOD to deal with the damage of ROS. However, the antioxidant capacity was limited, and the upper limit of this capacity was further reduced due to the effect of stress on enzyme synthesis. Moreover, the H_2O_2 and antioxidant enzyme activity of stems were lower than those of leaves. The sugar in the stems and leaves under LN treatment continued to accumulate, while more photosynthetic products were left in the leaves under HN treatment. As for K^+, it regulated the movement of sucrose to the sink organs through a complex mechanism called futile cycling. The analysis of PPO activity showed that its influence on the strength of the stem epidermis was limited and nondeterministic. To sum up, stem strength is mainly affected by cellulose and lignin, and their synthesis depends on the transportation of sucrose and the promotion of stem lignification by higher K^+ concentration, which shed light on the response of stem strength to stress. In addition, it should be noted that K^+ transport and transpiration are effective means for crops to adapt to abnormal K^+ supply within a certain range, which is also the reason for the lagging response of stem strength to K^+.

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