Pak. J. Bot., 56(3): 1099-1108, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-3(30)

EFFECTS OF THE NITROGEN APPLICATION RATE ON DRY MATTER ACCUMULATION, NITROGEN UPTAKE AND UTILIZATION, AND KEY ENZYME ACTIVITIES OF NITROGEN METABOLISM IN SORGHUM

MENG YANG LI[1], ZHEN QING GUO[1], YU XIN BAI[1], PENG FEI ZHU[1], JIAN WANG[1],
YU CUI HAN[1], FEI ZHANG[2*] AND XIAO HU LIN[1*]

[1]College of Agronomy and Biotechnology, Hebei Normal University of Science and Technology,
Qinhuangdao, Hebei 066004, China
[2]Institute of Sorghum, Liaoning Academy of Agricultural Sciences, Shenyang, Liaoning 110161, China
[*]Corresponding author's email: Xiaohulin2008@163.com; zhangfei19821121@163.com

Abstract

The effects of different nitrogen application rates (NAR) on sorghum yield and nitrogen (N) uptake and utilization in eastern Hebei Province, China were investigated. In this experiment, var. Liaoza 37 and var. Liaonian 3 were used as test materials, and 5 treatments were set up: 0 (N0), 120 (N1), 180 (N2), 240 (N3), 300 kg ha^{-1} (N4). The results showed that when NAR of 120 kg ha^{-1}, the maximum N accumulation of Liaoza 37 at maturity was 80.33 kg ha^{-1}. The average yield reached a maximum of 6631.43-6336.89 kg ha^{-1} when the NAR was 240-300 kg ha^{-1}. The results showed that when NAR of 180 kg ha^{-1}, the maximum N accumulation of Liaoza 37 at maturity was 80.80 kg ha^{-1}. The average yield reached a maximum of 6202.58-6610.07 kg ha^{-1} when the NAR was 180-240 kg ha^{-1}. Under high N conditions (240-300 kg ha^{-1}), nitrate reductase, glutamine synthetase, asparagine synthetase and nitrite reductase of Liaoza 37 maintained higher activities than those of Liaonian 3. Under low N conditions (120-180 kg ha^{-1}), nitrogen use efficiency (NUE), nitrogen agronomic efficiency (NAE), nitrogen absorption and utilization (NAAU), nitrogen partial production efficiency (NPPE), leaf N transport efficiency and leaf transfer volume contributed more N to the grain. Under high N condition (240-300 kg ha^{-1}), NAE, NAAU, leaf N transfer, leaf transfer efficiency, stem N transfer, stem transfer efficiency and leaf transfer volume contribution more N to the grain. Appropriately increasing the NAR helped to improve the transfer volume and transfer efficiency of the leaves and stems. A correlation analysis showed that the average yield of Liaoza 37 was positively correlated with the stem N transport efficiency and leaf ear N accumulation. The average yield of Liaonian 3 positively correlated with NAE, NAAU, leaf dry matter transfer efficiency and leaf dry matter transfer volume. These results suggested that there were significant differences in N uptake and the utilization of different sorghum varieties under different NAR. The study provided theoretical support for rational application of N fertilizer to sorghum varieties in eastern Hebei Province to obtain high yield.

Key words: Sorghum, Nitrogen metabolizing enzyme, Nitrogen use efficiency, Yield.

Introduction

N is an important component of proteins, amino acids, hormones, nucleic acids and other substances in plants (Liang, 2022; Mu & Chen, 2021; Bartzialis *et al.*, 2023). Since plants often require large amounts of N for normal growth, it is necessary to apply a large amount of additional N fertilizer to the soil in order to maintain high crop yields (Akinseye *et al.*, 2020; Tian *et al.*, 2018; Wei *et al.*, 2017). However, almost half of these N fertilizers input cannot be absorbed and utilized by plants, and the unutilized N fertilizers pose a huge threat to the environment and human health (Swoish & Steinke, 2017; Zhang *et al.*, 2019). Therefore, improving the NUF of plants while reducing N fertilizer input is a crucial aspect that requires urgent consideration (Qu *et al.*, 2020).

Sorghum bicolor (L.) Moench is the fifth largest crop in the world, with strong tolerance to low N (Khoddami *et al.*, 2023; Wagaw *et al.*, 2019; Zhang *et al.*, 2023). Sorghum roots can secrete biological nitrification inhibitors, which makes their NUE higher than other crops (Subbarao *et al.*, 2017; Tang *et al.*, 2018). Therefore, scientists are focusing on achieving optimal N fertilizer application and management in sorghum crops (Gao *et al.*, 2020; Su *et al.*, 2020). Although research on N utilization in sorghum has been widely reported, the results are inconsistent due to differences in regions and varieties. Tang *et al.*, (2018) reported that the optimal NAR was 60 kg ha^{-1} for sweet sorghum and 120 kg ha^{-1} for biomass sorghum. Almodares *et al.*, (2006) showed that sweet sorghum stalks, sucrose and grains planted in mid-may had the highest yields when N was applied at the 5-8 leaf stage. Schlegel *et al.*, (2021) demonstrated that the response of sorghum grain yield to N application could be optimized. From 1961 to 2015, it was found that the highest yield could be achieved when N was applied at 100 kg ha^{-1}. Therefore, under different environmental conditions, different sorghum varieties have different absorption and utilization of N due to genetic, evolutionary and physiological differences.

Numerous studies have been conducted on N accumulation, transport, and NUE in different sorghum varieties. Gao *et al.*, (2022) demonstrated that when the NAR was 160 kg ha^{-1}, the dry matter and N utilization rate of forage sorghum reached the maximum value, and the dry matter and NUE of forage sorghum showed a reducing trend with the continuous rise of NAR. Hassan *et al.,* (2023) reported that grain yield and dry matter accumulation of sorghum varieties with different genotypes were significantly different, and the advantages of *Rabin* and *Enkad* were both manifested in high biological fertilizer levels. However, there are few reports on the discussion of NAR for different sorghum varieties in the eastern Hebei region. Therefore, Liaoza 37 and Liaonian 3 were selected to investigate the effect of different NAR on the characteristics of dry matter accumulation, N uptake and utilization efficiency, and key enzyme activities of N assimilation pathway at different growth stages, with the intention of providing theoretical support for the rational application of N fertilizer in sorghum varieties.

Material and Methods

Overview of the experimental site: The experiment was conducted from 2021 to 2022 at the Experimental Station of Hebei Science and Technology Normal University (39°07'N, 119°17'E) in Changli County, Qinhuangdao City, located in the northeast of Hebei Province. The average annual sunshine is 2719.15 hours, the average annual rainfall is 527 mm, and the frost free period lasts up to 210 days. The soil type of the experimental site is medium loam., and the basic soil fertility of the 0-20cm soil layer is shown in Table 1.

Test materials and design: The experiment adopted a two factor split-plot design, with the main plot representing the NAR. Five NAR were used, namely 0 kg ha^{-1} (N0), 120 kg ha^{-1} (N1), 180 kg ha^{-1} (N2), 240 kg ha^{-1} (N3), and 300 kg ha^{-1} (N4); The sub-plots consisted of two sorghum varieties, including Liaoza 37 and Liaonian 3. The experimental area encompassed 21.6 m^2. Each treatment was replicated three times and the arrangment of treatments within the blocks was randomized. Urea, containing 46% N, was used as the N fertilizers, while superphosphate and potassium sulfate were employed as phosphorus and potassium fertilizers, respectively. The application rates for these fertilizers were 12% P_2O_5 (90 kg ha^{-1}) and 60% K_2O (108 kg ha^{-1}).

Sorghum varieties tested were obtained form the Sorghum Institute of Liaoning Academy of Agricultural Sciences. Liaoza 37 was sown on May 15, 2021, and harvested on September 10, 2021; Sown on May 18, 2022, and harvested on September 12, 2022. Liaonian 3 was sown on May 15, 2021, and harvested on September 10, 2021; Sown on May 18, 2022 and harvested on September 12, 2022. The use of well water irrigation, thorough cleaning of the straw after harvesting, and strict insect and diseases control measures well all implemented.

Table 1. Basic physicochemical properties of soil tested.

Year	Soil layer (cm)	Organi matter (g kg^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})	Alkali-hydrolyzed nitrogen (mg kg^{-1})
2021	0-20	31.28	25.35	113.34	125.18
2022	0-20	25.18	27.41	108.39	115.42

Measurement items and methods

Aboveground dry matter: At the mature stage of sorghum, three representative plants were selected from each plot to separate the leaves, petioles, ears, stems and grains. The fresh weight of the five parts was weighed by a balance with an accuracy of 0.01 g, put into kraft paper bags separately and kept in a 105℃ drying oven for 20 minutes, and dried them to constant weight at 80℃. Drying weight was recorded separately.

Determination of nutrients: After determining the aboveground dry matter, the dried samples were crushed and digested with H_2SO_4-H_2O_2. Subsequently, the total N content of various organs of the sorghum plant was determined using the Kjeldahl N analyzer method.

Measurement of yield and its constituent factors: Yield and its components were measured at the plant maturity stage. Plant samples were collected from three representative sample points (4 m^2) within each plot. After air drying, yield components such as plant number, grain number per panicle, spilkelet number per panicle, and thousand grain weight were determined.

Determination of enzyme activity related to nitrogen metabolism: The activities of enzymes involved in N metabolism were measured during the jointing stage (JS), flag leaf (FLS), and filling stages (FS). Leaf samples were taken from each treatment and stored in a -80℃ refrigerator for the determination of N metabolizing enzymes activities. The activities of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthetase (GOGAT), asparagine synthetase (AS), and nitrite reductase (NiR) were measured using the Keming Biological Reagent Kit. Enzyme activity was calculated according to kit instructions.

Parameters calculation: N accumulation (kg ha^{-1}) = Dry weight per plant at maturity × N content per plant during maturity;

Dry matter (N) transit volume (%) = Dry matter of various vegetative organs during flowering period (N) accumulated amount - Dry matter of various nutrient organs during maturity (N) sum of accumulated amounts;

$$\text{Dry matter (N) transit rate (\%)} = \frac{\text{Organ dry matter (N) transit volume}}{\text{Dry matter of vegetative organs during flowering period (N) accumulated amount}} \times 100$$

$$\text{Dry matter (N) contribution rate of transport volume to grains (\%)} = \frac{\text{Dry matter (N) transit volume}}{\text{Dry matter of mature grains (N) accumulated amount}} \times 100$$

$$\text{NUE (kg ha}^{-1}\text{)} = \frac{\text{Grain yield}}{\text{N accumulation in mature plants}}$$

$$\text{NAE (kg ha}^{-1}\text{)} = \frac{\text{Grain yield in N application area - Grain yield in areas without N application}}{\text{NAR}}$$

$$\text{NAAU (\%)} = \frac{\text{N accumulation in N application areas - N accumulation in non N application areas}}{\text{N rate}} \times 100$$

$$\text{NPPE (kg ha}^{-1}\text{)} = \frac{\text{Grain yield in N application area}}{\text{NAR}}$$

Statistical and analysis: Microsoft Excel (Microsoft Corp., Redmond, WA, USA) was used to process and plot experimental data, and SPSS 25.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software was used to perform analysis of variance (ANOVA). GGEBiplot installation package was used to perform correlation mapping based on the R language.

Results

Nitrogen accumulation: The total N accumulation in different organs of Liaoza 37 and Liaonian 3 reached its maximum values under N1 and N2 treatments, respectively (Fig. 1). Among aboveground parts of each plant variety, the N content was highest in the leaves, followed by ears, stems, petioles, and leaf ears. The N accumulation in the leaves of Liaoza 37 accounted for 33.27% to 38.85% of the total N accumulation per plant, and reached its maximum value in N1 treatment, which was remarkably higher than other treatments. The N accumulation in the leaves of Liaonian 3 accounted for 31.29% to 36.4% of the total N accumulation per plant and reached its maximum value in N2. N1 remarkably increased the N accumulation in the leaves of Liaoza 37 compared to N2, N3, and N4, by 15.18%, 15.71%, and 23.04%, respectively N2 significantly increased the N accumulation in the stem of Liaonian 3 compared to N0 and N4, by 18.57% and 20.00%, respectively. These results showed that under different N treatments, leaves were the main organs to accumulate N, and in Liaoza 37 and Liaonian 3, redistribution of N was promoted in the plants under N1 and N2 treatments, respectively.

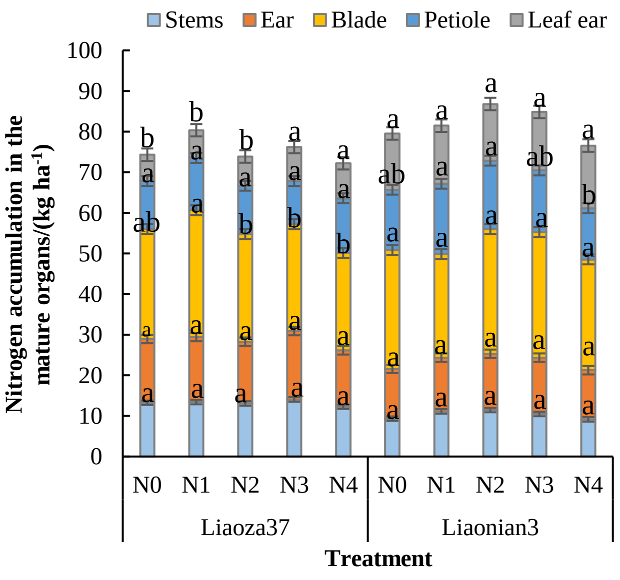

Fig. 1. Effects of nitrogen application rate on nitrogen accumulation in sorghum organs at maturity stage. Bars with different letters indicates significant difference at $p<0.05$.

Activity of key enzymes in nitrogen metabolism: Under the same NAR, the NR activity in both sorghum varieties showed an increasing trend from the JS to the FS (Fig. 2). With the increase of NAR, the NR activity of Liaoza 37 increased and reached the maximum under N4 treatment. It was remarkably higher than the N0 treatment at each growth stage, with an average increase of 34.78%-72.94% compared to N0. At the same reproductive period, with the increase of NAR, the trend of NR activity change in Liaonian 3 was not consistent. During the JS, the NR activity under N4 was remarkably higher than all other treatments. During the FLS, except for N2, the NR activity under N4 was remarkably higher than that of other treatments; During the FS, the NR activity under N4 was only remarkably higher than that of N0 treatment.

It can be seen from Fig. 2 that under the same N application, the GS activity in both the varieties showed a reducing trend from the JS to the FS. With the increase of NAR, the GS activity in Liaoza 37 was initially increased and then decreased. At each growth stage, the GS activity reached its maximum value under N3 treatment and was remarkably higher than N0 treatment. The average GS activity at each growth stage was 55.25%-75.54% higher than N0. With the increase of NAR, the trend of GS activity change in Liaonian 3 was not consistent. During the JS, except for N3, the GS activity under N4 was remarkably higher than all other treatments. During the FLS, the GS activity under N4 was only remarkably higher than that of N0. During the FS, under N4 was only significantly higher than N0.

As shown in Fig. 3, during the same growth period, the GOGAT activity in both varieties showed a trend of first rising and then reducing from the JS to the FS. During the JS, FLS and FS, the GOGAT activity in Liaoza 37 reached its maximum value under N3 treatment, significantly higher than N0 treatment by 48.01%, 25.87%, and 38.65%, respectively. During the JS, FLS and FS, the GOGAT activity in Liaonian 3 reached its maximum in N3, significantly higher than that of N0 by 61.49%, 69.18%, and 91.63%, respectively.

As shown in Fig. 3, under the same NAR, the AS activity in both varieties exhibited a decreasing trend from the JS to the FS. During the same reproductive period, with the increase of NAR, the trend of AS activity change in Liaoza 37 was not consistent. During the JS, except for N2, the AS activity under N4 treatment was remarkably higher than that of other treatments. During the FLS, the AS activity under N3 was remarkably higher than all other treatments; During the FS, the AS activity under N3 was remarkably higher than that of N0 and N1. During the same reproductive period, with the increase of NAR, the trend of AS activity change in Liaonian 3 was not consistent. During the JS, except for N1, the AS activity under N4 was much higher than that of other treatments. During the FLS and FS, the AS activity under N3 was remarkably higher than that of N0 and N1.

Under the same NAR, from the JS to the FS, Liaoza 37 showed a decreasing trend, while Liaonian 3 displayed first a rising and then a declining trend with respect to NiR activity (Fig. 4). During the same reproductive period, with the increase of NAR, NiR activity change in Liaoza 37 showed an inconsistent trend. During the JS, the NiR activity under N3 treatments was higher than that all other treatments. During the FLS, except for N4, the NiR activity under N3 was higher than that of other treatments. During the FS, the NiR activity under N0 was higher than that of N3 and N4. During the JS, FLS, and FS,the NiR activity in Liaonian 3 reached its maximum in N3, higher than that of N0 by 49.92%, 54.62%, and 58.45%, respectively.

Dry matter transport: The amount of N transported in the Liaoza 37 leaves showed a trend of first decreasing and then increasing with the increase of NAR, while in the stem it did not change significantly with varying N application (Fig. 5). The N transport in the leaves and stems of Liaonian 3 showed an rising trend with the increase of NAR. The N transport in the Liaoza 37 leaves under N0 treatment was higher than that of N1 and N3 treatments, manifested as N0 > N4 > N2 > N3 > N1. The N transport in the Liaonian 3 leaves reached its maximum value in N4 but was not significantly different compared to N0. The N transport in the Liaoza 37 stem under N4 was significantly higher by 31.68% compared to N1. The N transport in the Liaonian 3 stem under N4 was remarkably higher than all other treatments.

With the increase of NAR, the change in leaf transport efficiency of Liaoza 37 was not significant, while the stem transport efficiency showed an increasing trend. The N transport efficiency of the leaves and stems of Liaonian 3 showed an rising trend. The leaf transport efficiency of Liaoza 37 reached its maximum in N4 treatment, which was remarkably higher by 53.02% and 46.43% compared to N1 and N3 treatments, respectively. The transport efficiency of Liaonian 3 leaf reached its maximum value in N3, but was not different compared to N0. The stem transport efficiency of Liaoza 37 under N4 was higher than all other treatments. The stem transport efficiency of Liaonian 3 reached its maximum value in N4, which was remarkably higher by 40.05% and 38.83% compared to N0 and N1, respectively.

Nitrogen transport: The NUE of Liaoza 37 first showed an increase and then a reduction with the rise of NAR (Fig. 6). The NUE of Liaonian 3 showed a reducing trend with the rise of NAR. The NUE of Liaoza 37 under N2 treatment was significantly higher by 13.84% and 19.66% compared to N1 and N4 treatments, respectively, but was not significantly different compared to N0 treatment. The NUE of Liaonian 3 under N0 was remarkably higher than that under N4 by 15.89%. NAE in both Liaoza 37 and Liaonian 3 exhibited a trend of first rising and then reducing with the change of NAR. There is no significant difference in NAE among different treatments of Liaoza 37. NAE under N3 in Liaonian 3 was higher than that of N2 by 40.66%.

The NAAU of both Liaoza 37 and Liaonian 3 initially decreased and then increased with the increasing NAR. The NAAU of Liaoza 37 reached its maximum value in the N1 treatment and was remarkably higher than other treatments, demonstrated as N1 > N4 > N3 > N2. The NAAU of Liaonian 3 reached its maximum in N3, significantly higher by 37.93% compared to N2. The NPPE of Liaoza 37 and Liaonian 3 showed a reducing trend with the rise of NAR. Both Liaoza 37 and Liaonian 3 showed the maximum NPPE in N1, respectively, which was remarkably higher than that observed in other treatments.

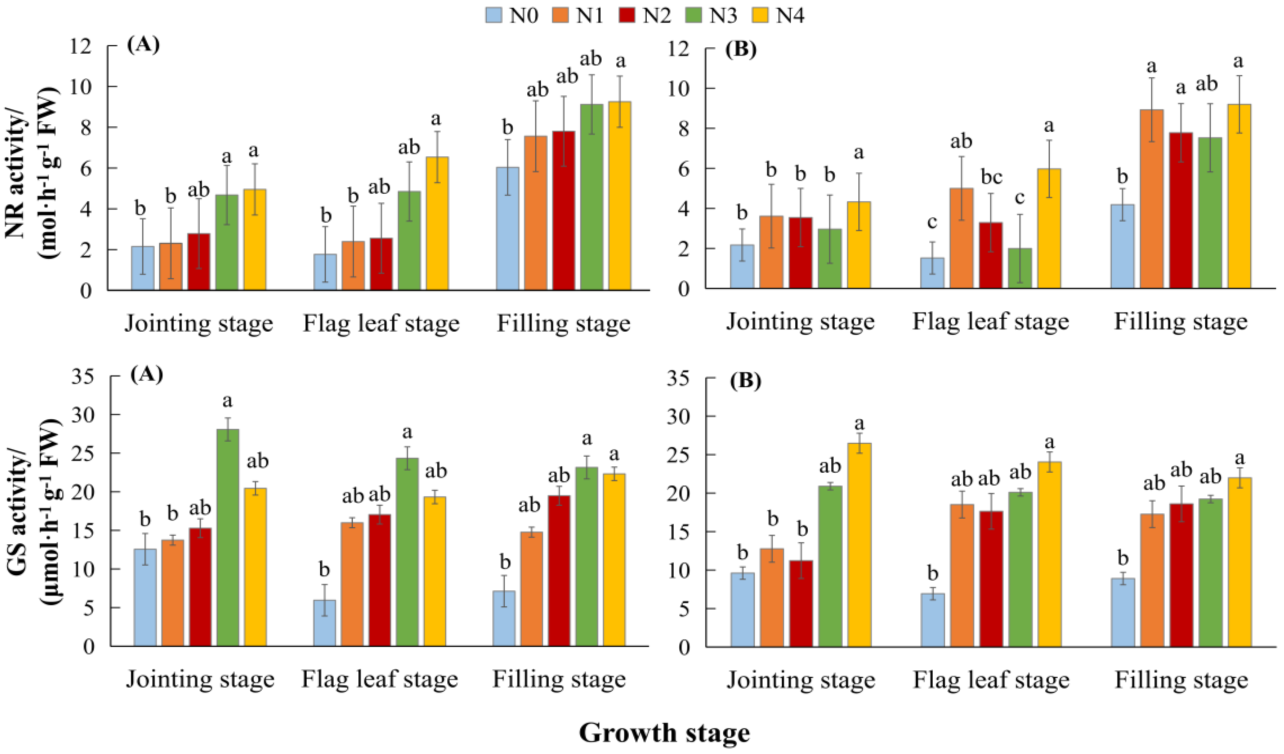

Fig. 2. Effect on nitrogen application rate on nitrate reductase and glutamine synthase in sorghum. (A) Liaoza 37; (B) Liaonian 3. Bars with different letters indicates significant difference at $p<0.05$. The same as following.

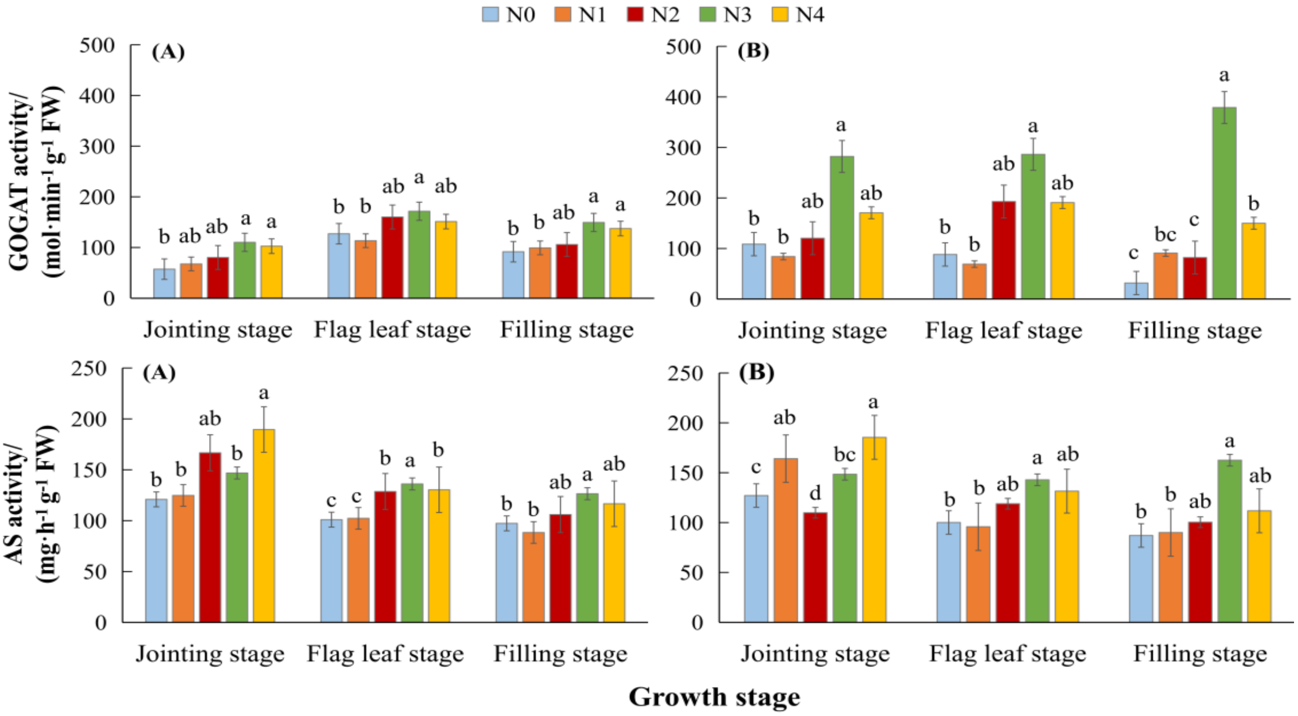

Fig. 3. Effect on nitrogen application rate on glutamate synthase and asparagine synthase in sorghum.

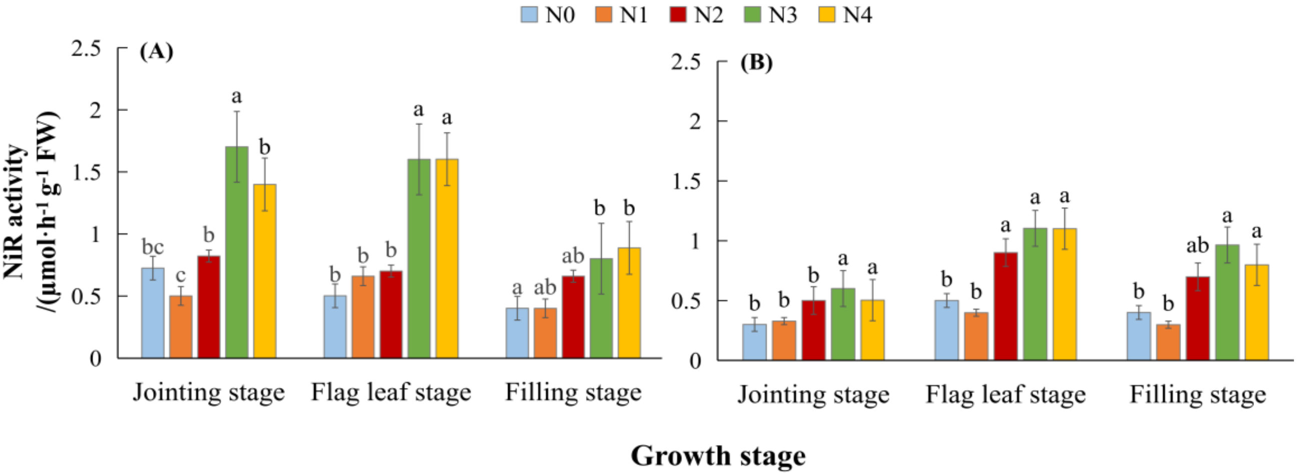

Fig. 4. Effect of nitrogen application rate on nitrite reductase in sorghum.

Nitrogen fertilizer efficiency: The N transport efficiency of Liaoza 37 leaves showed a trend of first decreasing and then increasing with the increase of NAR, whereas the N transport efficiency of stems did not change significantly as the NAR increased (Fig. 7). The N transport rate in the leaves and N transport efficiency in the stems of Liaonian 3 did not change remarkably with the increase of NAR. The N transport efficiency of the leaves of Liaoza 37 under N0 treatment was higher than that of N1, N2, and N3 treatments, manifested as N0 > N4 > N1 > N2 > N3. The N transport efficiency of Liaonian 3 leaves under N0 was higher than that of N1 and N4, demonstrated as N0 > N3 > N2 > N4 > N1. The N transport efficiency of the Liaoza 37 stem under N4 was higher than that of N0 and N2 by 61.84% and 30.55%, respectively. The N transport efficiency of the Liaonian 3 stem under N1 was significantly higher than that of N0 by 49.25%.

With the increase of NAR, the trend of leaf transport amount to grain contribution rate of Liaoza 37 was not consistent, while the trend of stem transport amount to grain contribution rate was first increasing and then decreasing. The contribution rate of leaf transport capacity to grain of Liaonian 3 showed a trend of first reducing and then rising, while the trend of stem transport capacity to grain contribution rate was not consistent. The contribution rate of leaf transport capacity to grains of Liaoza 37 reached its maximum value at N0 treatment, and was much higher than other treatments, manifested as N0 > N2 > N1 > N3 > N4. The contribution rate of leaf transport capacity to grain of Liaonian 3 reached its maximum value at N0, significantly higher by 25% and 15% compared to N2 and N3, respectively. The contribution rate of stem transport capacity to grains of Liaoza 37 and Liaonian 3 reached its maximum value in N3 and N1, respectively, and was remarkably higher than the other treatments.

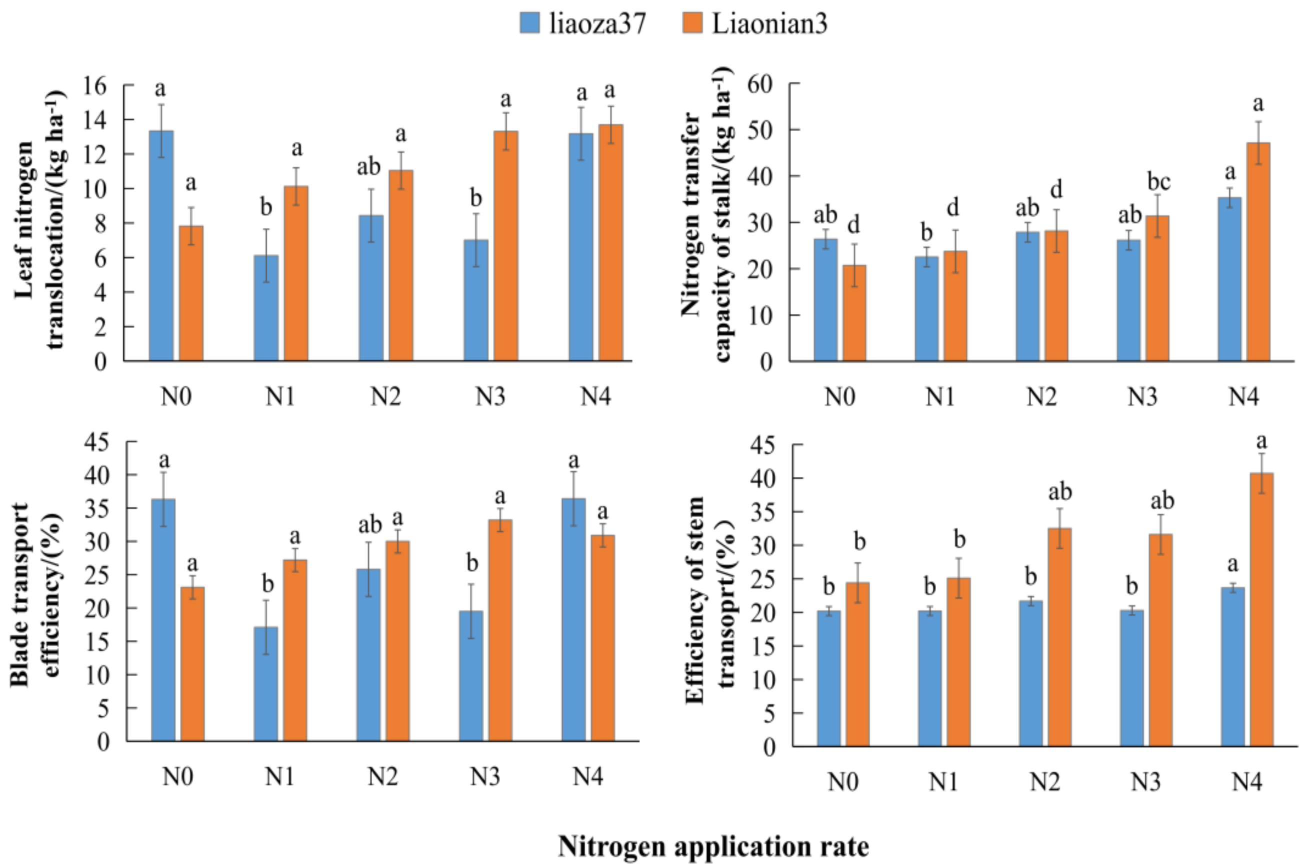

Fig. 5. Effect of nitrogen application rate on sorghum dry matter transfer volume. Bars with different letters indicates significant difference at $p<0.05$. The same as following.

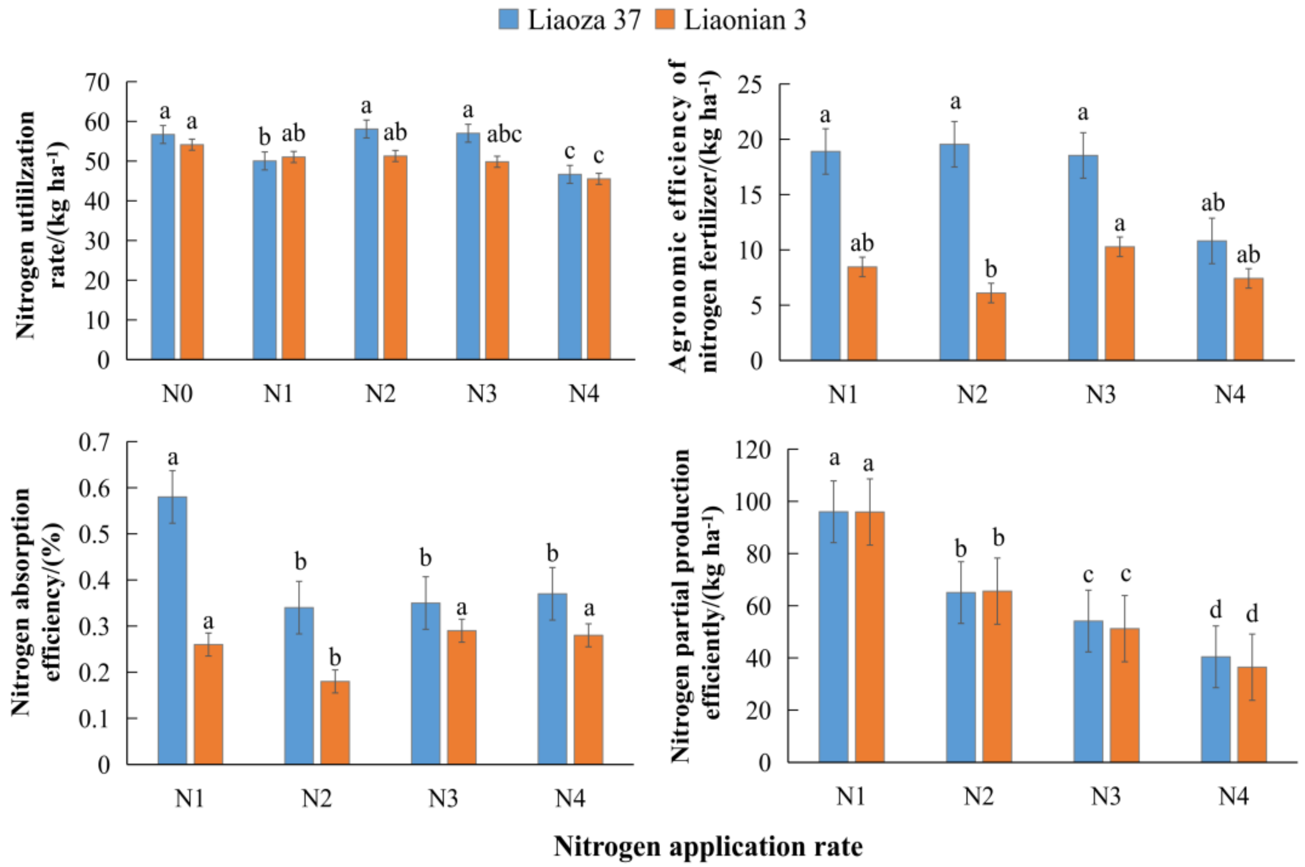

Fig. 6. Effect of nitrogen application rate on nitrogen transport in sorghum.

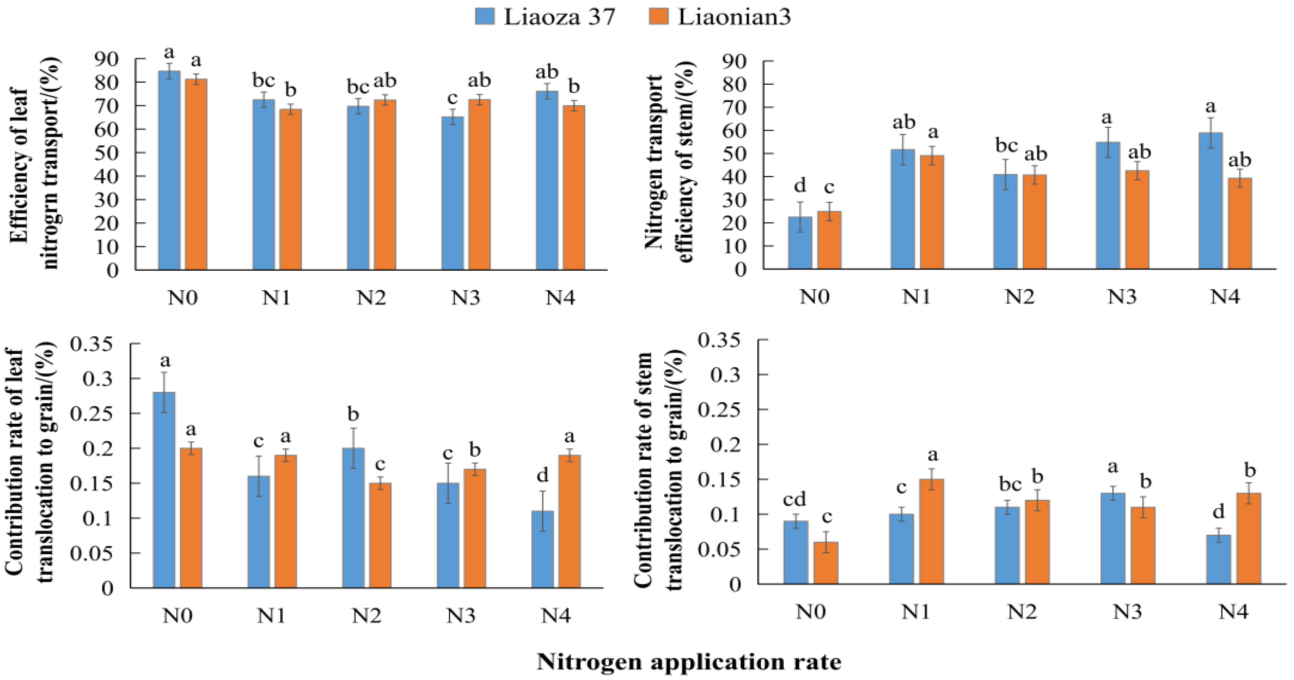

Fig. 7. Effect of nitrogen application rate on nitrogen efficiency of sorghum.

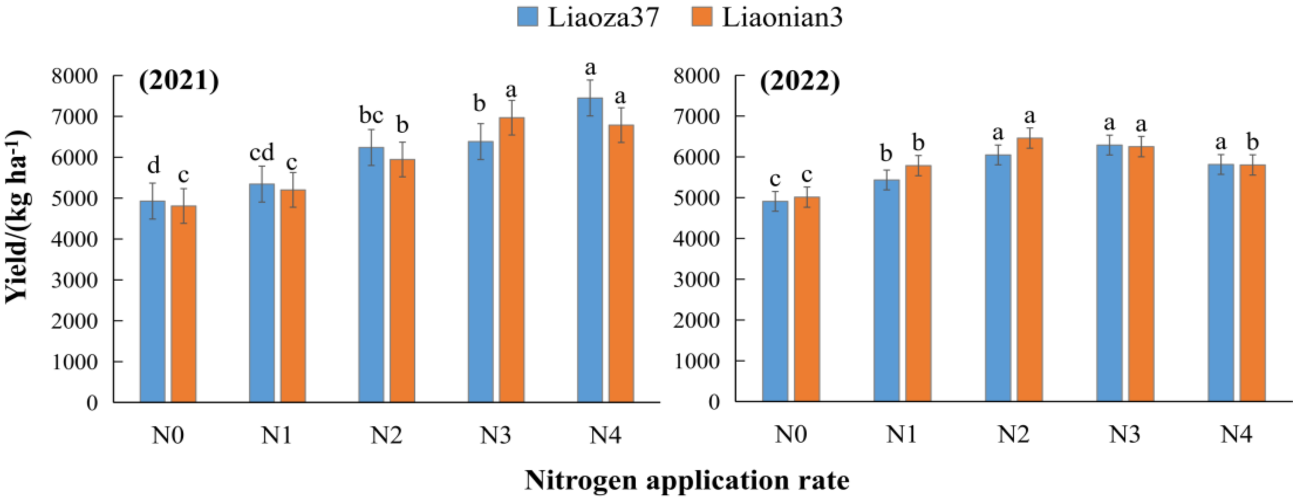

Fig. 8. Effect of nitrogen application rate on sorghum yield. Bars with different letters indicates significant difference at $p<0.05$.

Yield: The data from 2021 to 2022 indicate that different NAR significantly affected the yield of selected sorghum varieties, as shown in Fig. 8. In 2021, with the increase of NAR, the yield of Liaoza 37 was on the rise, while the yield of Liaonian 3 showed a trend of first rising and then reducing. The yield of Liaoza 37 ranged from 4927.4667 kg ha^{-1} to 7449.52 kg ha^{-1}, with a maximum yield of 7449.52 kg ha^{-1} in N4 treatment, which was remarkably higher than other treatments. The yield of Liaonian 3 ranged from 4808.1533 kg ha^{-1} to 6968.22 kg ha^{-1}. The maximum yield (6968.22 kg ha^{-1}) was observed under N3, which was remarkably higher than N0, N1, and N2. In 2022, the yields of Liaoza 37 and Liaonian 3 both initially rised and then reduced with the rise of NAR, reaching their maximum values in N3 and N2, respectively. The yield of Liaoza 37 under N3 was significantly higher by 21.94% and 13.61% compared to N0 and N1, respectively. Under N2, the yield of Liaonian 3 was significantly higher than that of N0, N1, and N4 by 22.4%, 10.43%, and 10.18%, respectively.

Correlation analysis: The relationship between the average yield and different parameters of Liaoza 37 and Liaonian 3 is shown in Fig. 9. The yield of Liaoza 37 was significantly positively correlated with AIL and NS (r = 0.814, 0.6220). The correlation between yield and AS, GOGAT enzyme activities was higher than that of NR, GS, and NiR enzyme activities. The yield was remarkably negatively correlated with LG and NIP (r = -0.556, -0.626). There was a positive correlation with OS, SD, AE, NAE, NF, indicating that the yield of Liaoza 37 was highly correlated with stem dry matter. The yield of Liaonian 3 was significantly positively correlated with OC, LD, AE, and NAE (r = 0.927, 0.882, 0.858, 0.825). The correlation between yield and NiR, GS enayme activities was higher than that of GOGAT, AS, NR activities. There was a significant negative correlation between yield and NUR (r = -0.782). Moreover, a significant positive correlation existed between AIL, SD, OS, SG, and NS, indicating a high correlation between the yield of Liaonian 3 and leaf dry matter.

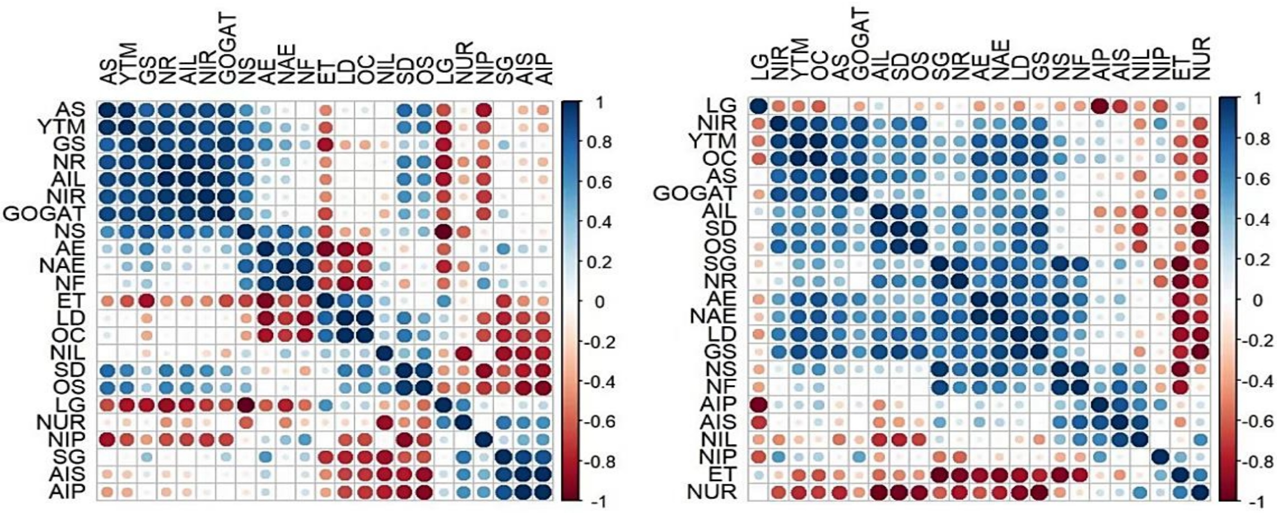

Fig. 9. The relationship between the average output of Liaoza 37 (left figure) and Liaonian 3 (right figure) from 2021 to 2022 and various indicators.

YTM: average production from 2021 to 2022; ET: efficiency of nitrogen transport in leaves; LG: leaf grain contribution rate; NS: efficiency of nitrogen transoprt in stem; SG: contribution rate of stem to grain; NUR: nitrogen utilization rate; AE: agronnmic efficiency of nitrogen fertilizer; NAE: nitrogen absorption utilization rate; NF: nitrogen fertilizer is more efficient; LD: leaf dry matter transfer; SD: stem dry matter transfer volume; OC: dry matter transport efficiency of le-aves; OS: stem dry matter transport efficiency; AS:asparagine synthase activity; NR:nitrate reductase activity; NIR: nitrite reductase activity; GS: glutamine synthase activity; GOGAT: glutamate synthase activity; AIS:effects of nitrogen accumulation in stem; AIP: effect of nitrogen accumulation in panicle; NIL: effect of petiole ni-trogen accumulation; NIP: effects of leaf nitrogen accumulation; AIL: effect of nitrogen accum-ulation in leaf ear.

Discussion

N application had obvious significance on dry matter accumulation and distribution in selected sorghum varieties. Leaf was the most important organ for N accumulation in both varieties, The total N accumulation in leaves of Liaoza 37 and Liaonian 3 accounted for 33.27%-38.85% and 31.29%-36.4% of the total N accumulation per plant, respectively, and increased by 7.5% and 10.45%, respectively as compared to N0 treatment. At the NAR of 300 kg ha^{-1}, the N accumulation of Liaoza 37 and Liaonian 3 decreased by 11.54% and 3.74%, respectively, compared to the N0 treatment, which was not conducive to N accumulation in different plant organs (Fig. 1). Rational application of N fertilizer affected the N content of sorghum organs, and improved the photosynthetic characteristics and metabolite transport to leaves, while excessive N application was subjected luxury N absorption phenomenon (Gura & Mnkeni, 2019*;* Heitman *et al.,* 2018). Low N application resulted in increased distribution to leaves, which was consistent with the results of low N fertilizer application.

N metabolism is a fundamental biological process, it significantly affects the protein synthesis in grains and other organs (Fang *et al.*, 2020; Zhang *et al.*, 2022). The processes of N accumulation, utilization and assimilation are regulated by the activities of key enzymes involved in N metabolism (Liu *et al*., 2021; Mattarozzi *et al*., 2020; Wu *et al.*, 2020). NR is a key enzyme that catalyzes the conversion of NO_3^- to NO_2^- in plants, playing an important role in regulating plant metabolism (Yoneyama *et al.*, 2019; Bailey *et al.*, 2019). This study found that the NR activity in both Liaoza 37 and Liaonian 3 reached its maximum values during the grouting period under N4 treatment (Fig. 2). This showed that higher NAR had a significant influence on NR activity, which was beneficial for N assimilation and accumulation. NiR is an intermediate enzyme in plants that catalyzes the conversion NO_2^- to NH_4^+ (Masclaux *et al.*, 2010; Srivastava *et al.*, 2018), playing a significant role in N utilization and assimilation. The NiR activity in both Liaoza 37 and Liaonian 3 reached its maximum values during the JS and FLS, respectively, at the N3 level, which was significantly different from the low N treatment (Fig. 4). It therefore implies that the low N treatment has a minimal impact on the enzyme activity of the two varieties, while higher N application levels result in higher enzyme activity. GS is an essential enzyme for converting inorganic N into organic N in plants. In both varieties, the GS activity reached its maximum value during the JS. Liaoza 37 exhibited the highest GS activity at the N3 level, whereas Liaonian 3 demonstrated its peak GS activity at the N4 level (Fig. 2). There is an important correlation between NAR and N metabolism enzyme activities in different sorghum varieties. In addition, the activities of GOGAT and AS enzymes are closely related to N accumulation. This study revealed that, from FLS to FS, Liaoza 37 could maintain the activities of GOGAT and AS only under high N levels (Fig. 3). Liaoza 37 required a higher NAR at the later stages of growth to complete grain and filling. Wang *et al.*, (2021) showed that increasing N application at appropriate rates can significantly improve the N metabolism in plants, resulting in a marked increase in yield. In this study, there was a significant positive correlation between N metabolism enzyme activities and stem N accumulation (Fig. 9). Sorghum plants were able to maintain high N metabolism enzyme activities during the middle to late grain FS, thereby improving the grains' capacity of converting and absorbing N. Overall the data indicated that the five enzyme activities in Liaoza 37 and Liaonian 3 showed a positive correlation with yield. Rational N application results in increased enzyme activities, high grain content, and hence the high yield (Akinseye *et al*., 2020; Lu *et al*., 2021; Nematpour *et al*., 2021).

Reasonable NAR can enhance photosynthetic and NUE in the plants (Shafiq *et al*., 2021; Tian *et al*., 2019). The fundamental aspect of scientific fertilizer application is the absorption and utilization of N fertilizer. There are many evaluation indicators to assess the ues efficiency of N fertilizer, among which the most commonly used indicators are N fertilizer agronomic utilization rate, N fertilizer absorption utilization rate, and N fertilizer partial productivity. These three indicators can evaluate the absorption and utilization of N and N fertilizer by crops from various aspects. Luo Kang *et al.,* (2021) demonstrated that moderate N application increased the dry matter quality and N accumulation in sorghum, while achieving higher NAE and NAAU. Gaspareto *et al.,* (2023) demonstrated that a NAR of 80 kg ha^{-1} could significantly increase the N accumulation in sorghum grains, the number of grains per panicles, and NUE. Samar *et al*., (2022) reported that a NAR of 200 kg ha^{-1} promoted the absorption of N in sorghum grains, stems, and improved total N content, grain yield, NPPE, and NUE. In the current study, the NAAU and NPPE of Liaoza 37 were significantly higher under low N treatment, compared to N4 treatment. However, with the increase of N application, NAAU and NPPE were decreased. At the moderate NAR, theNUE and NAE reached their highest values, and did not increase further with additional N4. The NUE and NPPE of Liaonian 3 were significantly higher than those at N4 level. At moderate NAR, the NUE and NAAU reached their highest values, and did not increase further with additional when N4 (Fig. 6). The average yield of the two varieties was positively correlated with NAE and NAAU (Fig. 9). This is consistent with the research results of Wang *et al*., (2022). This indicates that the N fertilizer input of Liaoza 37 and Liaonian 3 can be appropriately reduced, as excessive N application can cause N fertilizer loss and easily cause environmental pollution. The transportation amount and efficiency of dry matter in sorghum plants reached their highest values at higher NAR, which was consistent with the changes in yield, indicating that the transportation of dry matter promoted an increase in sorghum yield (Fig. 8). At high N levels, leaf transport capacity and leaf transport efficiency was of both Liaoza 37 and Liaonian 3 did not significantly increase compared to no N application, whereas stem transport capacity and stem transport efficiency significantly increased compared to no N application (Fig. 5). This indicates that higher N levels increased the transport capacity of sorghum stems, but had little effect on the transport capacity of leaves (Fig. 7). Excessive N application often leads to a decrease in nutrient use efficiency, causes soil nutrient loss, and also has negative impact on the environment. These findings suggest that, rational use of N fertilizer can promote the absorption and utilization of N in sorghum (Nematpour *et al*., 2021), improve NUE and hence, contribute to achieving high yield of sorghum.

Conclusion

The results showed that there were significant differences in N uptake and use efficiency between Liaoza 37 and Liaonian 3. The results showed that when NAR of 120 kg ha^{-1}, the maximum N accumulation of Liaoza 37 at maturity was 80.33 kg ha^{-1}. The average yield reached a maximum of 6631.43-6336.89 kg ha^{-1} when the NAR was 240-300 kg ha^{-1}. The results showed that when NAR of 180 kg ha^{-1}, the maximum N accumulation of Liaoza 37 at maturity was 80.80 kg ha^{-1}. The average yield reached a maximum of 6202.58-6610.07 kg ha^{-1} when the NAR was 180-240 kg ha^{-1}. Under high N conditions (240-300 kg ha^{-1}), nitrate reductase, glutamine synthetase, asparagine synthetase and nitrite reductase of Liaoza 37 maintained higher activities than those of Liaonian 3. In both sorghum varieties, moderate NAR remarkably increased the N content of sorghum leaves and stems, the transport capacity and efficiency of leaves and stems are improved, promoted the accumulation of N in the leaves and stems, and simultaneously increased the sorghum yield and nutritional value. Moderate application of N fertilizer can effectively delay the aging process of leaves, improve dry matter distribution, and increase sorghum yield. This study provides a theoretical basis for the high production and rational application of N fertilizer for two important sorghum varieties (Liaoza 37 and Liaonian 3) of the eastern Hebei region.

Acknowledgement

This work was supported by National "13th Five-Year Plan" key Research and Development Plan Project-Grain quality and efficiency varieties screening and supporting cultivation technology (2019YFD1001701-2), Discipline Performance Project of Liaoning Academy of Agricultural Science (2022-HBZ-1008) and Hebei Province Modern Agriculture Industry Technology System Innovation Team (Miscellaneous grain and beans) Project (HBCT2023050404). Thanks are also to four anonymous reviewers for their thoughtful and valuable comments and suggestions, which is helpful in improving the manuscript.

References

Akinseye, F.M., H.A. Ajeigbe, P.C. Traore, S.O. Agele, B. Zemadim and A. Whitbread. 2020. Improving sorghum productivity under changing climatic conditions: A modelling approach. *Field Crop Res.,* 246: 107685.

Almodares, A. and S.M.M. Darany. 2006. Effects of planting date and time of nitrogen application on yield and sugar content of sweet sorghum. *J. Environ. Biol.,* 27(3): 601-605.

Bailey, S.J., J.E. Parker, E.A. Ainsworth, G.E.D. Oldroyd and J.I. Schroeder. 2019. Genetic strategies for improving crop yields. *Nature*, 575: 109-118.

Bartzialis, D., K.D. Giannoulis, I. Gintsioudis and N.G. Danalatos. 2023. Assessing the efficiency of different nitrogen fertilization levels on sorghum yield and quality characteristics. *Agric.*, 13(6): 1253.

Fang, H., X. Gu, T. Jiang, J. Yang, Y. Li, P. Huang, P. Chen and J. Yang. 2020. An optimized model for simulating grain-filling of maize and regulating nitrogen application rates under different film mulching and nitrogen fertilizer regimes on the Loess Plateau, China. *Soil. Till. Res.*, 199: 104546.

Gao, F., J. Hu, B.Z. Ren, P. Liu, B. Zhao and J.W. Zhang. 2020. Improving soil properties and grains yield of winter wheat and summer maize under residue management strategies. *Agro. J.,* 112(5): 4287-4302.

Gao, W., N. Shou, C.Z. Jiang, R.S. Ma, Y.Y. Shen and X.L. Yang. 2022. Effect of nitrogen application rate on dry matter accumulation, distribution, and water use efficiency of forage sorghum. *Acta Prataculturae Sinica,* 31(9): 26-35. (In Chinese)

Gaspareto, R.N., A. Jalal, W.C.N. Ito, C.E.D.S. Oliveira, C.M.D.P. Garcia, E.H.M.A. Boleta, P.A.L. Rosa, F.S. Galindo, S. Buzetti, B.B. Ghaley and M.C.M.T. Filho. 2023. Inoculation with plant growth-promoting bacteria and nitrogen doses improves wheat productivity and nitrogen use efficiency. *Microorganism,* 11(4): 1046.

Gura, I. and P.N.S. Mnkeni. 2019. Crop rotation and residue management effects under no till on the soil quality of a haplic cambisol in Alice, eastern cape, South Africa. *Geoderm.*, 337: 927-934.

Hassan, A.A., A.S. Hussein and A.Z.S.A. Al-Hassan. 2023. Effect of different levels of bio fertilizer on growth and yield of sorghum genotypes. *Iop Conference Series: Earth Environ. Sci.*, 1158(6): 062019.

Heitman, A.J., M.S. Castillo, T.J. Smyth and C.R. Crozier. 2018. Stem, leaf, and panicle yield and nutrient content of biomass and sweet sorghum. *Agro. J.*, 110(5): 1659-1665.

Khoddami, A., V. Messina, K.V. Venkata, A. Farahnaky, C.L. Blanchard and T.H. Roberts. 2023. Sorghum in foods: functionality and potential in innovative products. *Crit. Rev. Food Sci.*, 63: 1170-1186.

Liang, G.P. 2022. Nitrogen fertilization mitigates global food insecurity by increasing cereal yield and its stability. *Glob Food Secur.*, 34: 100652.

Liu, X., W. Gu, C. Li, J. Li and S. Wei. 2021. Effects of nitrogen fertilizer and chemical regulation on spring maize lodging characteristics, grain filling and yield formation under high planting density in Heilongjiang province, China. *J. Integ. Agri.*, 20: 511-526.

Lu, X.F., E.Q. Hou, J.Y. Guo, F.S. Gilliam, J.L. Li, S.B. Tang and Y.W. Kuang. 2021. Nitrogen addition stimulates soil aggregation and enhances carbon storage in terrestrial ecosystems of China: A meta-analysis. *Global Change. Biol.*, 27(12): 2780-2792.

Luo K., Y.J. Zeng., Q.H. Shi, W.S. Lv, X.B. Xie, L. Guo, C. Cheng and Q.C. Zhou. 2021. Study on the effects of nitrogen application rate and density on the yield and nitrogen utilization efficiency of double crop rice with machine direct seeding. *J. Nucl. Agri. Sci.*, 35(12): 2850-2859. (In Chinese)

Masclaux, D.C., F.V. Daniel, J. Dechorgnat, C. Fabien, G. Laure and S. Akria. 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Ann. Bot.*, 105(7): 1141-1157.

Mattarozzi, M., J.D. Zinno, B. Montanini, M. Manfredi, E. Marengo, F. Fornasier, A. Ferrarini, M. Careri and G. Visioli. 2020. Biostimulants applied to maize seeds modulate the enzymatic activity and metapro-teome of the rhizosphere. *Appl. Soil. Ecol.*, 148(4): 103480.

Mu, X.H. and Y.L. Chen. 2021. The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiol. Bioch.*, 158: 76-82.

Nematpour, A., H.R. Eshghuzadeh and M. Zahedi. 2021. Comparing the corn, millet and sorghum as silage crops under different irrigation regime and nitrogen fertilizer levels. *Int. J. Plant Prod.*, 15(3): 351-361.

Qu, Z.M., X.C. Qi, R.G. Shi, Y.J. Zhao, Z.P. Hu, Q. Chen and C.L. Li. 2020. Reduced N fertilizer application with optimal blend of controlled-release urea and urea improves tomato yield and quality in greenhouse production system. *J. Soil. Sci. Plant Nut.*, 20(1): 1741-1750.

Samer, S., A. Dov ile., M. Romas and B. Zita. 2022. Influence of modified urea compounds to improve nitrogen use efficiency under corn growth system. *Sustainability*, 14 (21): 14166.

Schlegel, A. and J. Havlin. 2021. Irrigated grain sorghum response to 55 years of nitrogen, phosphorus, and potassium fertilization. *Agro. J.*, 113: 464-477.

Shafiq, I., S. Hussain, M.A. Raza, N. Iqbal, M.A. Asghar, A. Raza, Y.F. Fan, M. Mumtaz, M. Shoaib, M. Ansar, A. Manaf, W.Y. Yang and F. Yang. 2021. Crop photosynthetic response to light quality and light intensity. *Integ. Agri.*, 20(1): 4-23.

Srivastava, R.K., R.K. Panda, A. Chakraborty and D. Halder. 2018. Enhancing grain yield, biomass and nitrogen use efficiency of maize by varying sowing dates and nitrogen rate under rainfed and irrigated conditions. *Field Crop Res.*, 221: 339-349.

Su, Y., M. Yu, H. Xi, J.L. Lv, Z.H. Ma, C.L. Kou and A.L. Shen. 2020. Soil microbial community shifts with long-term of different straw return in wheat-maize rotation system. *Sci. Pep.*, 10(1): 293-301.

Subbarao, G.V., J. Arango, K. Masahiro, A.M. Hooper, T. Yoshihashi, Y. Ando, K. Nakahara, S. Deshpande, I. Ortiz-Monasterio, M. Ishitani, M. Peters, N. Chirinda, L. Wollenberg, J.C. Lata, B. Gerard, S. Tobita, I.M. Rao, H.J. Braun, V. Kommerell, J Tohme and M. Iwanaga. 2017. Genetic mitigation strategies to tackle agricultural GHG emissions: the case for biological nitrification inhibition technology. *Plant Sci*., 262: 165-168.

Swoish, M.K. and K. Steinke. 2017. Plant growth regulator and nitrogen applications for improving wheat production in Michigan. *Crop Forage. Turf. Man.*, 3(1): 1-7.

Tang, C.C., X.L. Yang, X. Chen, A. Ameen and G.H. Xie. 2018. Sorghum biomass and quality and soil nitrogen balance response to nitrogen rate on semiarid marginal land. *Field Crop Res.*, 2: 12-22.

Tian, J., C.L. Wang, J.L. Xia, L.S. Wu, G.H. Xu, W.H. Wu, D. Li, W.C. Qin, X. Han, Q.Y. Chen, W.W. Jin and F. Tian. 2019. Teosinte ligule allele narrows plant architecture and enhances high-density maize yields. *Science*, 365(6454): 658-664.

Tian, Z.W., X.X. Liu, S.L.Gu, J.H.Yu, L. Zhang, W.W. Zhang, D. Jiang, W.X. Cao and T.B. Dai. 2018. Postponed and reduced basal nitrogen application improves nitrogen use efficiency and plant growth of winter wheat. *J. Integr. Agric*., 17(12): 2648-2661.

Wagaw, K. 2019. Review on mechanisms of drought tolerance in sorghum (*Sorghum bicolor* (L.) Moench) basis and breeding methods. *Acad. Res. J. Agric. Sci. Res.*, 7: 87-99.

Wang, H.Q., Y.H. Huang, G.Y. Jiang, Y.T. Liu and Z.Q. Che. 2022. Effect of nitrogen fertilizer base dressing ratio on nitrogen metabolism and nitrogen fertilizer utilization efficiency of spring wheat under drip irrigation. *J. Soil. Water Conser.*, 36(1): 297-306, 315. (In Chinese)

Wang, J.F., Z.Z. Wang, F.X. Gu, F.X. Mou, Y. Wang, J.Z. Duan, W. Feng, Y.H. Wang and T.C. Guo. 2021. Effects of nitrogen density regulation on carbon and nitrogen metabolism and yield of two winter wheat varieties. *Chin. Agric. Sci.*, 54(19): 4070-4083.

Wei, S.S., X.Y. Wang, Q.C. Zhu, D. Jiang and S. Dong. 2017. Optimizing yield and resource utilization of summer maize under the conditions of increasing density and reducing nitrogen fertilization. *Sci. Nat.*, 104: 11-12.

Wu, K., S.S.Wang, W.Z. Song, J.Q. Zhang, Y. Wang, Q. Liu, J.P. Yu, Y.F. Ye, S. Li, J.F. Chen, Y. Zhao, J. Wang, X.K. Wu, M.Y. Wang, Y.J. Zhang, B.M. Liu, Y.J. Wu, N.P. Harberd and X.D. Fu. 2020. Enhanced sustainable green revolution yield via nitrogen-responsive chromatin modulation in rice. *Acad. Res. J. Agri. Sci. Res.*, 367: 1-9.

Yoneyama, T. and A. Suzuki. 2019. Exploration of nitrate-to-glutamate assimilation in non-photosynthetic roots of higher plants by studies of ^{15}N-tracing, enzymes involved, reductant supply, and nitrate signaling: A review and synthesis. *Plant Physiol. Bloch.*, 136(3): 245-254.

Zhang, F., J.X. Wang, K.Y. Zhang, H. Wu, F.L. Ke, Y.H. Duan, Y.Q. Wang, J.Q. Zhou, K. Zhu, Z.P. Zhang, F. Lu and H.T. Zhou. 2022. Regulation of photosynthetic material production by inter-root microbial extinction and metabolic pathways in sorghum under different nitrogen application patterns. *Sci. Pep.*, 12(1): 6755.

Zhang, R.D., Z.X. Yue, X.F. Chen, R.D. Huang, Y.F. Zhou and X. Cao. 2023. Effects of waterlogging at different growth stages on the photosynthetic characteristics and grain yield of sorghum (*Sorghum bicolor* L.). *Sci. Pep.*, 13: 7212.

Zhang, S.Q., L. Yuan, W. Li, Z.A. Lin, Y.T. Li, S.W. Hu and B.Q. Zhao. 2019. Effects of urea enhanced with different weathered coal-derived humic acid components on maize yield and fate of fertilizer nitrogen. *J. Integ. Agri.*, 18(3): 656-666.

(Received for publication 5 April 2023)