LRF-723
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RESEARCH ARTICLE Legume Research- An International Journal, Volume Issue : ()

Dynamic Response of Nitrogen Content to Moisture Variation in Soybean during the Whole Growth Period

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10.18805/LRF-723

ABSTRACT

Background: Soybeans are the main sources of oil and protein for most of the global population. As the population grows, so does the demand for soybeans. However, drought is a major factor that limits soybean growth.

Methods: The nitrogen content in different parts of the soybean under drought stress and rehydration was investigated using the soybean cultivars, HN44 and HN65, at different growth stages (vegetative growth, parallel period of vegetative growth and reproductive growth and reproductive growth).

Result: During the vegetative growth stage, drought decreased the plant's nitrogen content. After rehydration, all the organs showed different degrees of compensatory effects. During the parallel period of vegetative and reproductive growth, drought decreased the nitrogen content of the leaves. After rehydration, the nitrogen content was partially restored. During the reproductive growth period, drought affected the translocation of nitrogen to the sink. After rehydration, the nitrogen content increased in the pods and decreased in other parts. In summary, rehydration after drought during the vegetative growth period of soybean can lead to nitrogen accumulation, but the reproductive growth phase differs from earlier; therefore, this period should avoid drought stress.

Key words: Compensatory effect, Drought stress, Nitrogen content, Soil water content, Soybean.

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How to cite this article: Wang, X., Tian, Y., Liu, S., Wang, X., Wu, Z. and Dong, S. (2023). Dynamic Response of Nitrogen Content to Moisture Variation in Soybean during the Whole Growth Period. Legume Research. doi: 10.18805/LRF-723.

Submitted: 27-10-2022 **Accepted:** 09-01-2023 **Online:** 25-03-2023

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is an essential crop that has seeds rich in protein and oil and is of considerable importance in food production and daily life (Jassal and Singh, 2020). Nitrogen plays a major role in the growth and yield of soybeans and is mainly derived from nitrogen in the soil and N_2 in the air. Two forms of inorganic nitrogen (nitrate and ammonium salts) are absorbed from the soil via the root system (Hachiya and Sakakibara, 2017). In contrast, organic nitrogen is absorbed directly by the root system and accounts for a relatively small fraction of the overall nitrogen content (Bloom, 2015).

Globally, agricultural production consumes a large quantity of water; however, drought is one of the major factors limiting crop cultivation (Neha and Sanjeev, 2017) and this problem is likely to intensify as global warming worsens (Sabina and Sameena, 2022). Drought is an environmental stressor that often affects plant growth by interfering with the absorption of ions and water, thereby hindering nitrogen metabolism and severely impacting plant growth (Godwin and Farrona, 2020). Previous studies have found that rehydration after drought stress in some periods may increase crop biomass and cause the growth rate to exceed that of crops that are not dehydrated. This phenomenon is known as the compensatory effect (Huang, 2000). However, most relevant studies have focused on the impacts of drought on crop nitrogen metabolism, while few have reported the integration of the nitrogen content of drought rehydrated crops.

In this study, we selected the drought-tolerant and drought-sensitive varieties HN44 and HN65, to investigate the changes in nitrogen under different degrees of drought and rehydration. Our findings provide a reference for future studies on soybean drought and its compensatory effects. Moreover, understanding the various changes in water demand during crop growth stages can better combine regional climate and hydrological conditions, which is of great significance for coping with environmental changes and increasingly serious soil-moisture fluctuations.

MATERIALS AND METHODS

Soybean varieties used in this study were Heinong 44 (drought resistant type; HN44) and Heinong 65 (sensitive type; HN65). The seeds were provided by The Soybean Research Institute of Heilongjiang Academy of Agricultural Sciences (Harbin, China). Drought resistance or sensitivity can be distinguished according to the survival rate after drought at the seedling stage (Wang *et al.*, 2022).

The experiment began in late spring (May) of 2021 and lasted until early autumn (September) at the Northeast

Agricultural University. The monthly average air humidity was 51% in May, 65% in June, 77% in July, 78% in August and 70% in September. The changes in the temperature are shown in Fig 1. The meteorological data were measured by the Northeast Agricultural University Meteorological Station. Plastic barrels with a diameter of 26 cm and a height of 33 cm were used. Each barrel contained 12 kg of air-dried soil; the soil base total nitrogen content was 1.42 g/kg and the total phosphorus content was 0.62 g/kg. The total potassium content was 25.53 g/kg and the organic matter content was 23.52 g/kg. Full grain soybean seeds were selected for sowing and three seedlings were planted in each pot. The soil moisture measuring instrument ECH2O-TE/EC-TM (EM-50, Decagon, Washington DC, USA) was used to control water and drought stress was applied during the vegetative growth period (V3), parallel period of vegetative growth and reproductive growth (R2) and reproductive growth phase (R5). Drought stress was categorized as sufficient water supply (soil water content was 70-75% of the field water holding capacity), mild stress (60-65%), moderate stress (50-55%) and severe stress (30-35%).

For the control group, adequate water supply was maintained throughout the growth stages of the plants. In the processing group, the water supply was interrupted when the soybean plant entered specific periods (V3, R2 and R5 stages), causing it to dry, after which the soil moisture content of the treatment group was measured daily. When the soil moisture content reached a predetermined range, the plants were randomly sampled to form the drought treatment group. Rehydration of the remaining drought plants was carried out to restore soil water content to control levels, one week after rehydration and random samples were selected as the drought rehydration treatment group. This was repeated three times for each process whilst ensuring rain protection to eliminate the effects of natural rainfall on the moisture content of the soil throughout the experiment.

The plant tissues (leaves, stems and petioles) were heated at 105°C for 30 min, dried at 65°C for 96 h and crushed. The dried plant tissues were then analyzed for the total nitrogen content using the Kjeldahl method (Gao and Wu, 2012).

All data were processed using Microsoft Office Excel 2021, statistical analysis was performed using IBM SPSS software (version 23.0; IBM Corporation, Armonk, NY, USA) and figures were produced using OriginPro 2021 (Origin Lab Corp., Northampton, MA, USA) software.

RESULTS AND DISCUSSION

Effect of drought and rehydration in vegetative growth period on nitrogen content

As shown in Fig 2, in the V3 stage, the total nitrogen content in the leaves and petioles of HN44 decreased under drought stress. No significant differences were observed between the stems. After rehydration, the total nitrogen in the leaves under mild drought rehydration showed a significant compensatory effect, with an increase of 4.8% and the petioles, similarly, showed a compensatory effect. The total nitrogen content in the stems after rehydration was still lower than that of the control. Under drought stress at the V3 stage, the variable trend of the total nitrogen content of HN65 was similar to that of HN44. The change in nitrogen content in the stems was not significant, but in the leaves and petioles, it decreased significantly. The nitrogen content in the leaves of HN65 plants decreased significantly by 12% under mild drought. In comparison, the nitrogen content of HN44 was only reduced by 5% under the same circumstances,

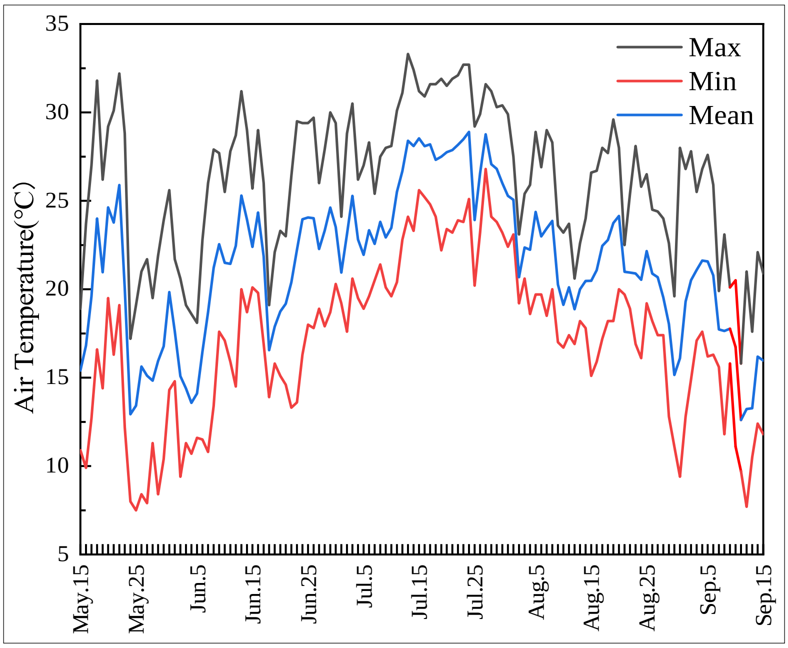

Fig 1: Maximum, minimum and average temperature of the experimental site from May 15 to September 15, 2021.

indicating that drought during this period had a greater impact on the nitrogen level of HN65. After rehydration, all organs of HN65 showed a compensatory effect compared to the control, but there was no significant difference under different degrees of drought rehydration treatment. The compensatory effect of HN65 after rehydration was higher than that of HN44, indicating that the compensatory effect was more significant for varieties that were greatly affected by drought.

A certain degree of abiotic stress can induce a compensatory effect in crops, which is reflected in their yield or growth state (Balducci *et al.*, 2016). Our study further confirms this view, because nitrogen also shows a compensating effect after drought rehydration. During the vegetative growth period, the total nitrogen content in stems did not change significantly, whereas that in leaves and petioles decreased, indicating that during drought stress, the nitrogen in leaves was transferred to roots through the phloem. This conclusion was supported by the results of Shi *et al.* (2020). Song *et al.* (2019) showed that nitrogen content affects the drought tolerance of maize and on comparing this with our results, we concluded that nitrogen transport may be a mechanism for plants to adapt to drought. The findings of Parabha *et al.* (2018) confirmed this conclusion.

Effect of drought and rehydration in parallel period of vegetative growth and reproductive growth on nitrogen content

Fig 3 shows that in the R2 stage, the total nitrogen content in all organs of HN44 showed a downward trend and the nitrogen in leaves decreased the most under mild stress by 22.7%. After rehydration, the nitrogen content in all organs of HN44 under mild drought rehydration were lower than that of the control, but nitrogen loss was alleviated. A compensatory effect is evident after moderate and severe stress rehydration. The nitrogen content of HN65 also decreased after drought stress at the R2 stage, but the decrease was much smaller than that of HN44, which reflects the difference between the two varieties under drought stress. From the perspective of nitrogen content, HN65 performed better than HN44. After rehydration, the amplitude of compensatory mechanism gradually decreased with an increase in stress.

During the R2 stage, the nitrogen absorbed by the roots is transported to the leaves and then distributed to new leaves and flowers (Tegeder and Masclaux-Daubresse, 2018). Under drought stress, the nitrogen content decreased more than that observed in the V3 stage. Therefore, this served as a reflection of the significance of the water demand during this time. Previous studies have revealed that the plants during flowering period are water-sensitive (Faralli *et al.*, 2017), therefore, ensuring water availability during this time is crucial for production (Gol *et al.*, 2021). When the leaves were rehydrated, their nitrogen concentration increased in a manner reminiscent of the V3 stage. Because vegetative growth still occurs during this period, excessive accumulation of nitrogen occurs (El-Nakhlawy *et al.* 2018). However it was still unable to reverse the damage to flowers during drought. Studies have shown that water stress during pollen development can inhibit meiosis and spore formation in microsporocytes (Lamin-Samu *et al.*, 2021), which is the basic reason for reduction in the yield when drought occurs during this period (Yu *et al.*, 2019). Concurrently, there was a decrease in nitrogen in the stems and petioles, which may be a unique change during this period, which we believe is unfavorable.

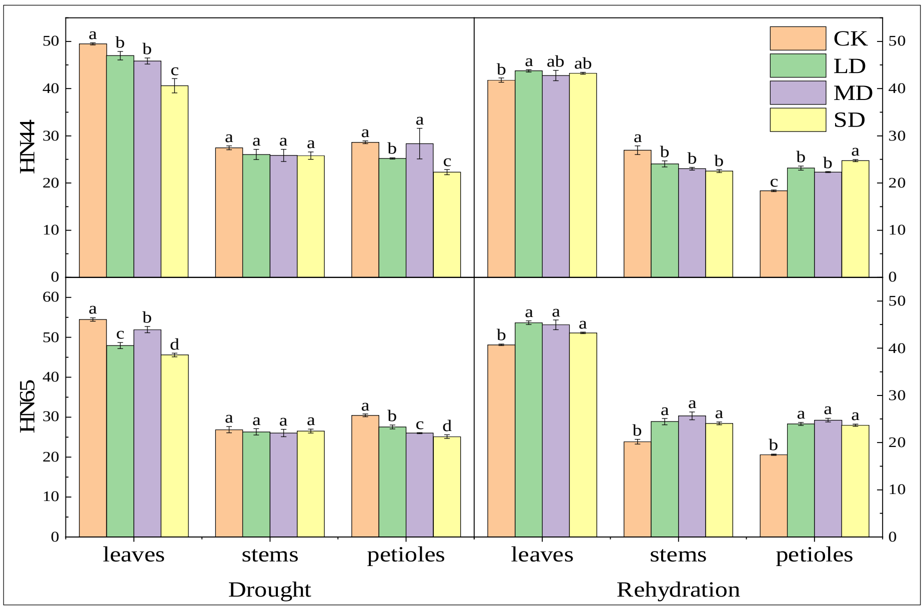

Fig 2: Effect of water change on total nitrogen content (mg/g) in vegetative growth period (V3) (Significance analysis was carried out between different degrees of stress in the same organ. P<0.05).

Effect of drought and rehydration in the reproductive growth phase on nitrogen content

A new sink organ-pod appeared in soybean during the reproductive growth phase. Therefore, drought in this period will have a more complex impact on plants. As shown in Fig 4, under drought stress, the total nitrogen content in the leaves and petioles of HN44 was not significantly affected and in stems it increased, whereas in pods it decreased. The nitrogen content decreased by up to 6.2% in pods under severe stress. This indicates that drought affects the transport of nitrogen. After rehydration, the nitrogen content in the leaves, stems and petioles decreased, whereas the nitrogen content in the pods increased.

In HN65, drought stress significantly decreased the nitrogen content in stems, petioles and pods. The nitrogen content of the pods gradually decreased with increasing drought stress. After rehydration, the nitrogen content in the leaves was lower than that in the control, whereas in the stems, it increased by 34.4% after severe stress rehydration.

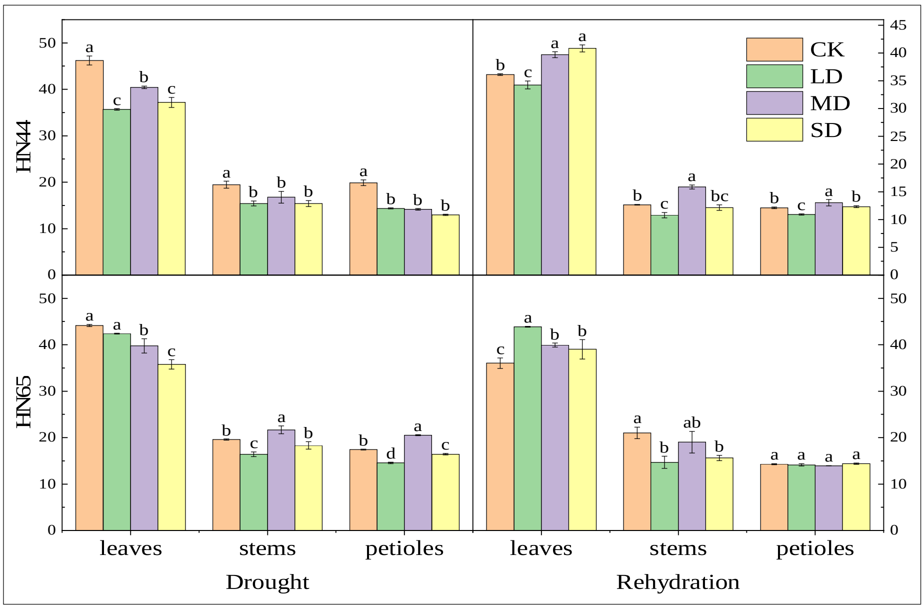

Fig 3: Effect of water change on total nitrogen content (mg/g) in Parallel period of vegetative growth and reproductive growth (R2) (Significance analysis was carried out between different degrees of stress in the same organ. P<0.05).

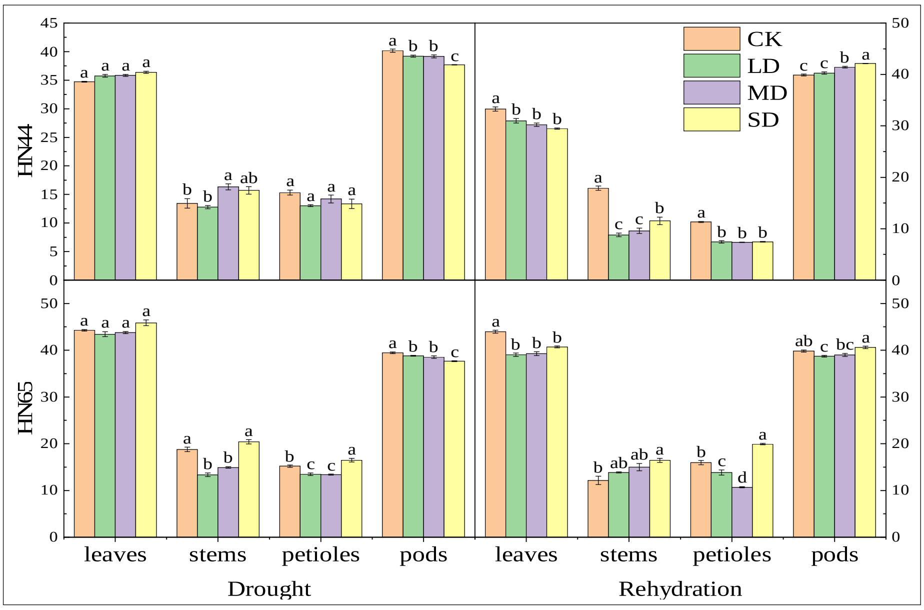

Fig 4: Effect of water change on total nitrogen content (mg/g) in generative growth phase (R5) (Significance analysis was carried out between different degrees of stress in the same organ. P<0.05).

After entering the reproductive growth stage, nutrients are transported to pods to form seeds. This period is critical for yield formation (Shao *et al.*, 2021). Water status plays an important role in nutrient transport. The main causes of yield loss during this period are premature senescence and lodging caused by water stress (Farooq *et al.,* 2017), which means that substances such as amino acid and carbohydrates in the leaves are decomposed and transported and thus leaf loses its biological function (Yu *et al.*, 2022). Crop yield is a continuous process that requires the continuous transport of nutrients to grains (Mitchell *et al.*, 2020). In this study, it was found that the normal redistribution of nitrogen was blocked under drought. After rehydration, the nitrogen transport disorder caused by water deficit was alleviated and nitrogen was supplied to the pods.

Nitrogen transport under water change in whole growth period

To show the change in nitrogen content in plants after drought and rehydration more clearly, a heat map was drawn (Fig 5). It can be seen from the map that the drought at the V3 stage led to a decrease in nitrogen in the leaves and

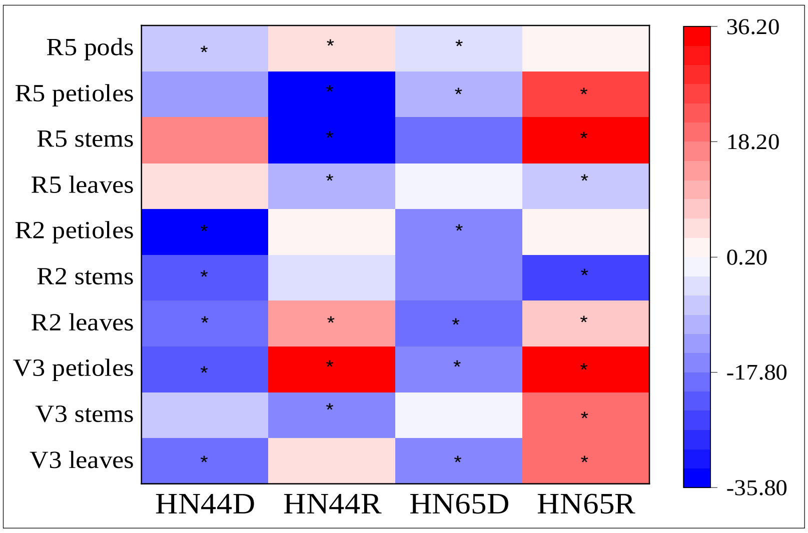

Fig 5: Heat map based on percentage change in drought and rehydration compared to control. The map using severe drought and severe drought–rehydration data. The "*" represents a significant difference compared with the control. P<0.05.

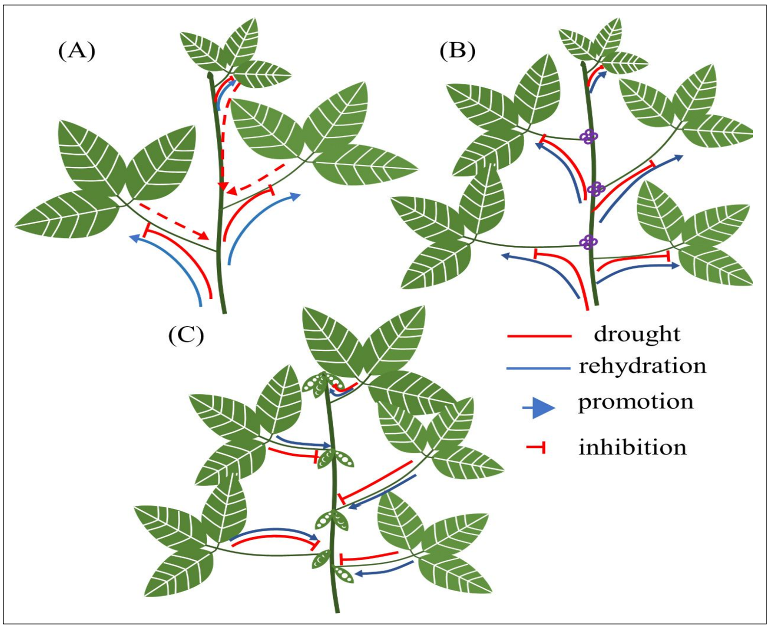

Fig 6: Nitrogen transport diagram (A); Nitrogen Transport in Vegetative Growth Period (B); Nitrogen Transport in the Period of Vegetative Growth and Reproductive Growth (C); Nitrogen transport during reproductive growth Period. The dotted line in (A) represents the inference process.

recovered after rehydration, indicating that the transportation of nitrogen is susceptible to water. The process of nutrient transport depends on the collection of xylem and leaf transpiration is the main driving force of the process (Kunrath *et al.*, 2020). As shown in figure 6A, drought stress affected nitrogen transport during vegetative growth. After recovery from stress, a compensatory effect was observed in the stems and leaves. Therefore, when the seedlings are in the vegetative growth stage, appropriate drought (not enough to kill the plants) followed by rehydration could have a positive effect on plant growth. A similar finding was reported by Poveda *et al.* (2018).

At the R2 stage, nitrogen was transported from the roots to the leaves and then redistributed to the flowers and new leaves. As shown in figure 6B, under drought stress, this period was similar to V3 performance, indicating that the nitrogen transport process was blocked. After rehydration, the nitrogen content in the leaves increased, indicating that the plants had increased the absorption and transport of nitrogen.

During the reproductive growth stage (R5), leaf redistribution is the main source of nitrogen in the pods (Taniguchi *et al.*, 2018). After drought treatment, the nitrogen content in the pods of HN44 decreased significantly, while the remaining parts did not change significantly, indicating that the nitrogen flow in the plant was stagnant and the process of leaf redistribution to the pod was significantly inhibited (Fig 6C). This effect is relieved by rehydration.

CONCLUSION

Drought and rehydration had significant effects on soybean nitrogen content. Drought stress in the vegetative growth stage leads to a decrease in nitrogen content; however, after rehydration, it shows a compensatory effect of nitrogen. During the parallel period of vegetative and reproductive growth, drought led to a decrease in the nitrogen content of the leaves. After rehydration, the nitrogen content was partially restored. During the reproductive growth stage, drought hinders the transport of nitrogen to pods, affecting yield formation, which is alleviated after rehydration. Therefore, in the process of agricultural production, drought stress should be avoided in parallel periods of vegetative and reproductive and reproductive growth period of crops. In contrast, drought stress during the vegetative growth period has a positive effect, provided that this stress does not cause crop death.

Funding

The research was funded by Natural Science Foundation of Heilongjiang Province of China, grant number LH2021C023.

Conflict of interest: None.

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