

## Article

# Effect of Nitrogen Supply on Growth and Nitrogen Utilization in Hemp (*Cannabis sativa* L.)

Yang Yang , Wenxin Zha, Kailei Tang , Gang Deng , Guanghui Du and Feihu Liu \*

Laboratory of Plant Improvement and Utilization, Yunnan University, Kunming 650500, China; yjy@ynu.edu.cn (Y.Y.); wsy198797@163.com (W.Z.); kailei.tang@ynu.edu.cn (K.T.); denggang1986@ynu.edu.cn (G.D.); dgh2012@ynu.edu.cn (G.D.)

\* Correspondence: dmzpynu@126.com

**Abstract:** Hemp is a multipurpose crop that is cultivated worldwide for fiber, oil, and cannabinoids. Nitrogen (N) is a key factor for getting a higher production of hemp, but its application is often excessive and results in considerable losses in the soil–plant–water continuum. Therefore, a rational N supply is important for increasing N efficiency and crop productivity. The main objective of this paper was to determine the responses of four hemp cultivars to different levels of exogenous-N supply as nutrient solution during the vegetative growing period. The experiment was conducted at Yunnan University in Kunming, China. Yunma 1, Yunma 7, Bamahuoma, and Wanma 1 were used as the experimental materials, and five N supplying levels (1.5, 3.0, 6.0, 12.0, and 24.0 mmol/L NO<sub>3</sub>-N in the nutrient solution) were set by using pot culture and adding nutrient solution. The root, stem, and leaf of the plant were sampled for the determination of growth indexes, dry matter and N accumulation and distribution, and physiological indicators. The plant height, stem diameter, plant dry weight, and plant N accumulation of four hemp cultivars were significantly increased with the increase in exogenous-N supply. Root/shoot dry weight ratios, stem mass density, and N use efficiency decreased significantly with the increase in exogenous-N supply. Nitrogen accumulation, chlorophyll content, soluble protein content, and nitrate reductase activity in leaves were increased with the increase in exogenous-N supply. Among the four indexes, the increase in N accumulation was more than the increase in NR activity. The activities of superoxide dismutase and peroxidase in leaves were increased first and then decreased with the increase in exogenous-N supply, with the maximum value at N 6.0 mmol/L, while the content of malondialdehyde in leaves increased significantly when the level of exogenous-N supply exceeded 6.0 mmol/L. These results revealed that increasing the exogenous-N supply could improve the plant growth, dry matter accumulation, and N accumulation in hemp during the vegetative growth period, but N supply should not exceed 6.0 mmol/L. Among four hemp cultivars, Wanma 1 performed well at 6.0 mmol/L N application.

**Keywords:** hemp (*Cannabis sativa* L.); nitrogen nutrition; nitrogen utilization efficiency; vegetative growth



**Citation:** Yang, Y.; Zha, W.; Tang, K.; Deng, G.; Du, G.; Liu, F. Effect of Nitrogen Supply on Growth and Nitrogen Utilization in Hemp (*Cannabis sativa* L.). *Agronomy* **2021**, *11*, 2310. <https://doi.org/10.3390/agronomy11112310>

Academic Editors: Christos Noulas, Shahram Torabian and Ruijun Qin

Received: 15 October 2021

Accepted: 13 November 2021

Published: 15 November 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

As an important component of macromolecular substances such as proteins, nucleic acids, phospholipids, hormones, and chlorophyll in plants, nitrogen (N) is one of the essential macronutrients for plant growth and development. Sufficient N supply is an important factor to improve crop yield and quality [1]; however, an overdose of N fertilizer will lead to the decrease in fertilizer efficiency in the plant and cause a large amount of N loss that gives rise to a series of environmental issues [2,3]. Therefore, the rational application of N fertilizer is one of the key measures for the high yield and effective cultivation of crops.

Hemp (*Cannabis sativa* L.) is a multipurpose crop. Cannabinoids and other secondary metabolites extracted from hemp inflorescence and leaves are widely used in medical treatment, beauty, and health care. The multipurpose utilization of hemp significantly improves

its economic value and planting [4–6]. The high-yield and high-efficiency cultivation mode of hemp has also become one of the hot spots in hemp production [7–9]. N is the macronutrient absorbed by hemp, and sufficient N supply can greatly improve the stem weight and leaf biomass in hemp [10,11]. Papastylianou et al. [12] reported that hemp biomass yield, stem dry weight, and inflorescence weight increased by 37.3%, 48.2%, and 16%, respectively, with the application of 240 kg N ha<sup>-1</sup> when compared with the unfertilized control. It is easy to diagnose and mitigate N deficiencies during the hemp-growing season, while excessive N is difficult to diagnose. Therefore, farmers prefer to apply N fertilizer in excess in attempts to increase the yield. Nevertheless, the excessive N fertilizer not only impedes increase hemp productivity but also reduces hemp yield caused by plant lodging and serious pests and diseases. According to studies conducted in the Latvia and Western Canada, N fertilization rates ranged between 50 and 200 kg N ha<sup>-1</sup>, while the highest bast fiber yields were obtained at N rates between 50 and 150 kg N ha<sup>-1</sup> [13,14]. Amaducci et al. [15] reported that 100 kg ha<sup>-1</sup> of N was the natural availability in the soil, and each additional kg of N supplied via fertilization increased hemp stem dry matter production by 20 kg but increased plant mortality. Therefore, it is very important to understand the utilization capacity of exogenous-N for carrying out the high-yield and high-efficiency cultivation of hemp.

A plethora of research reported the utilization of exogenous-N supply in hemp under field conditions. However, due to the difficulty of precise regulation of the N nutrient status in the field, there is still a lack of deep understanding in this research area. Previous work has shown that hemp is sensitive to N fertilizer during the vegetative phase by absorbing 88.2% of the total amount N in the whole growth period, which directly affects the accumulation of plant biomass [16]. Thus, the present study was conducted on hemp using pot culture and irrigation of nutrient solution with two specific objectives i.e., (i) to assess the utilization capacity of exogenous-N during the vegetative growing period in hemp; and (ii) to investigate the effects of excessive N on the growth and physiological attributes of hemp. The results will contribute to the understanding of hemp response to an environment with excessive N supply.

## 2. Materials and Methods

In this study, four commercial domestic hemp cultivars were selected as the experimental materials. Of them, Yunma 1 (YM1, fiber-type) and Yunma 7 (YM7, fiber-type) were provided by the Yunnan Academy of Agricultural Sciences, Bamahuoma (BM, seed-type) was provided by the Guangxi Academy of Agricultural Sciences, and Wanma 1 (WM1, fiber-type) was provided by Lu'an Agricultural Science Research Institute of Anhui Province.

The pot experiment was conducted in a greenhouse of Yunnan University, Kunming, China. The seeds were sown in 30 cm × 20 cm (diameter × depth) plastic pots filled with 1.2 kg peat (dry weight). In this kind of peat, total N, total P, total K, hydrolyzable N, Olsen phosphorus, and available potassium were 5.46, 0.37, 1.04, 0.14, 0.14, and 0.42 g kg<sup>-1</sup>, respectively, which were measured by using standard methods. Before sowing, peat was irrigated to 30% peat water content. Three weeks after sowing, ten uniform hemp plants were left per pot after thinning. A half liter of nutrient solution was applied every three days in each pot and lasted seven weeks. In the nutrient solution, N concentration was set at five levels as 1.5, 3.0, 6.0, 12.0, and 24.0 mmol/L NO<sub>3</sub>-N. The nutrient solution was based on Hoagland formula with modifications as KCl replacing KNO<sub>3</sub>, and CaCl<sub>2</sub> supplementing Ca<sup>2+</sup> for the low N level solutions. Three replicates for each N level were implemented in the experiment.

Seven weeks after treatment with different N levels, ten plants were sampled from each pot. Five plants were used to determine the growth-related indexes and N accumulation (NA), and the remaining five plants were used to measure the contents of chlorophyll (Chl), soluble protein (SP), and malondialdehyde (MDA) as well as the activity of superoxide dismutase (SOD), peroxidase (POD), and nitrate reductase (NR).

After measuring plant height and stem diameter, plants were divided into root (flushed and washed with running water), leaf, and stem; these parts were placed in an oven, first at 108 °C for 20 min and then at 80 °C until drying out. The dry weights (DW) of the root, leaf, and stem were recorded, and then, the average weight per plant was calculated. The dried root, stem, and leaf were pulverized and sieved; then, they were digested with H<sub>2</sub>O<sub>2</sub>-H<sub>2</sub>SO<sub>4</sub>. The total nitrogen content (TN) was determined by the Kjeldahl method [17]. The indicators were calculated according to the formulas listed below:

Total plant DW (DWP, g/plant) = root DW + stem DW + leaf DW;

Ratio of root to shoot = root DW / (stem DW + leaf DW);

Stem mass density (SMD, g/cm<sup>3</sup>) = stem DW / stem volume;

NA of plant part (g/plant) = plant part DW × TN;

NA per plant (NAP, g/plant) = root NA + stem NA + leaf NA;

Nitrogen use efficiency (NUE, g/g) = DWP / NAP.

Fully expanded leaves were separated from the upper part of the other five plants. Leaf laminae without midrib were mixed for determining physiological indexes (Chl, SP, MDA, SOD, and POD) using the methods described by Wang et al. [18]. NR activity was measured following the protocol described by Silveira et al. [19].

The data were handled fundamentally by Excel 2016 software. Standard deviation was calculated for each treatment in each cultivar. Effects of N levels on the growth indexes, N accumulation, and physiological indices were tested with one-way ANOVA followed by Duncan's test at  $p < 0.05$  (SPSS 23.0).

The 'R' was used to determine the Pearson's correlation coefficients (<https://cran.r-project.org/web/packages/Hmisc/index.html>, 12 November 2021) and for a heatmap (<https://www.r-graph-gallery.com/heatmap/>, 12 November 2021).

### 3. Results

#### 3.1. Effect of N Supply on Plant Growth

The plant height and stem diameter of hemp cultivars were increased with the increase in exogenous-N level, reaching the maximum value at 24.0 mmol/L, while the root/shoot dry weight ratio and SMD showed a contrary pattern. Plant height and root/shoot weight ratio did not change significantly when the N concentration was 12.0 mmol/L or more; the plant height of YM1 and SMD of WM1 did not change significantly when the N concentration was 6.0 mmol/L or more; the SMD of YM7 showed a significant difference among N concentrations (Table 1).

**Table 1.** Effect of N treatments on the hemp growth parameters.

Cultivar	Nitrogen (mmol/L)	Plant Height (cm)	Stem Diameter (mm)	Root/Shoot Ratio	Stem Mass Density (g/cm <sup>3</sup> )
YM1	1.5	62 ± 8 c	3.3 ± 0.2 c	0.37 ± 0.03 a	0.90 ± 0.09 a
	3.0	75 ± 5 b	3.5 ± 0.3 c	0.36 ± 0.03 ab	0.78 ± 0.09 b
	6.0	89 ± 2 a	4.6 ± 0.4 b	0.33 ± 0.02 b	0.40 ± 0.01 c
	12.0	94 ± 5 a	5.0 ± 0.5 b	0.28 ± 0.01 c	0.36 ± 0.02 cd
	24.0	95 ± 1 a	6.1 ± 0.5 a	0.28 ± 0.02 c	0.29 ± 0.01 d
BM	1.5	45 ± 2 d	3.9 ± 0.2 b	0.43 ± 0.03 a	0.82 ± 0.04 a
	3.0	54 ± 2 c	4.2 ± 0.5 b	0.39 ± 0.06 ab	0.73 ± 0.23 b
	6.0	68 ± 1 b	4.7 ± 0.5 ab	0.35 ± 0.07 abc	0.50 ± 0.21 b
	12.0	70 ± 4 ab	4.7 ± 0.5 ab	0.31 ± 0.02 bc	0.49 ± 0.16 b
	24.0	72 ± 1 a	5.2 ± 0.7 a	0.30 ± 0.06 c	0.43 ± 0.18 b
YM7	1.5	58 ± 4 d	3.1 ± 0.4 d	0.36 ± 0.02 a	1.14 ± 0.09 a
	3.0	71 ± 7 c	4.0 ± 0.5 c	0.32 ± 0.02 b	0.61 ± 0.05 b
	6.0	81 ± 3 b	4.4 ± 0.4 c	0.29 ± 0.01 c	0.51 ± 0.04 c
	12.0	87 ± 4 ab	5.2 ± 0.4 b	0.28 ± 0.01 c	0.40 ± 0.01 d
	24.0	92 ± 1 a	6.1 ± 0.4 a	0.28 ± 0.01 c	0.30 ± 0.03 e

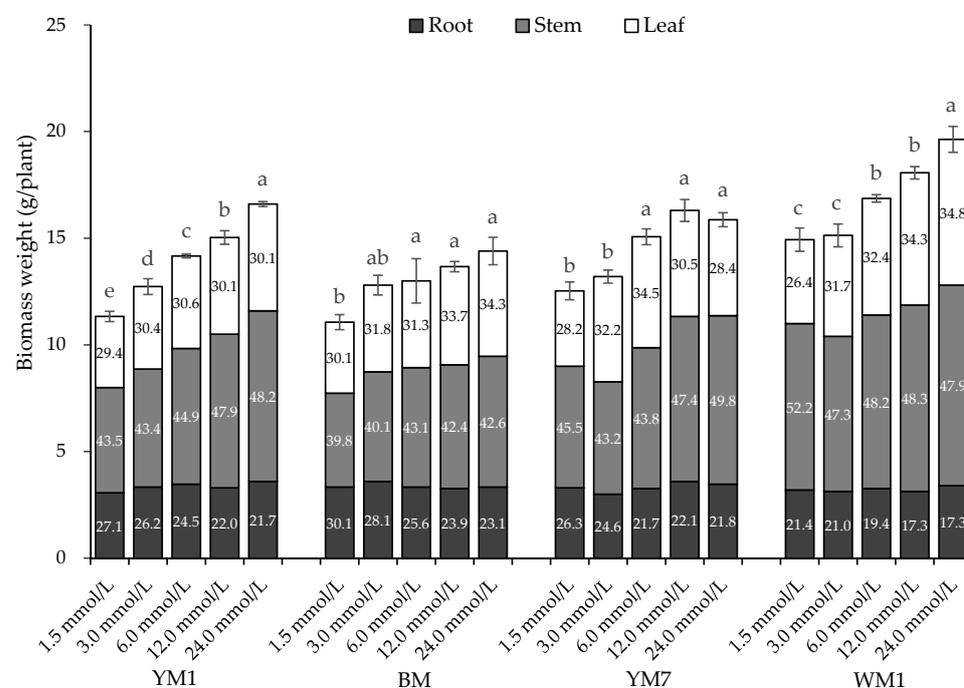
Table 1. Cont.

Cultivar	Nitrogen (mmol/L)	Plant Height (cm)	Stem Diameter (mm)	Root/Shoot Ratio	Stem Mass Density (g/cm <sup>3</sup> )
WM1	1.5	69 ± 6 c	3.2 ± 0.2 d	0.27 ± 0.02 a	1.42 ± 0.04 a
	3.0	76 ± 2 c	3.5 ± 0.2 c	0.26 ± 0.01 ab	0.96 ± 0.17 b
	6.0	90 ± 4 b	4.6 ± 0.2 b	0.24 ± 0.02 b	0.55 ± 0.05 c
	12.0	98 ± 6 ab	4.8 ± 0.3 ab	0.21 ± 0.02 c	0.50 ± 0.06 c
	24.0	104 ± 4 a	5.0 ± 0.1 a	0.21 ± 0.01 c	0.47 ± 0.02 c
YM1	1.5	62 ± 8 c	3.3 ± 0.2 c	0.37 ± 0.03 a	0.90 ± 0.09 a
	3.0	75 ± 5 b	3.5 ± 0.3 c	0.36 ± 0.03 ab	0.78 ± 0.09 b
	6.0	89 ± 2 a	4.6 ± 0.4 b	0.33 ± 0.02 b	0.40 ± 0.01 c
	12.0	94 ± 5 a	5.0 ± 0.5 b	0.28 ± 0.01 c	0.36 ± 0.02 cd
	24.0	95 ± 1 a	6.1 ± 0.5 a	0.28 ± 0.02 c	0.29 ± 0.01 d

Different letters following the numbers within columns represent significant difference at  $p = 0.05$  within a cultivar. Hemp cultivars: Yunma 1 (YM1), Bamahuoma (BM), Yunma 7 (YM7), Wanma 1 (WM1).

### 3.2. Effect of N Supply on Hemp Biomass

The biomass (total plant dry weight) of YM7 was increased first and then decreased with the increase in N concentration. A maximum value of dry weight was found at 12.0 mmol/L, while in other cultivars, it was increased along with the N supply levels, reaching the maximum value at 24.0 mmol/L; the biomass of YM7 and BM did not change significantly when the N concentration was 6.0 mmol/L or more. Among plant parts, with the increase in N concentration, the dry weights of stem and leaf were increased greatly, but this increase in root dry weight increased was very little, and the root/total plant dry weight ratio decreased continuously (Figure 1). Among four hemp cultivars, WM1 performed well at 6.0 mmol/L N application.

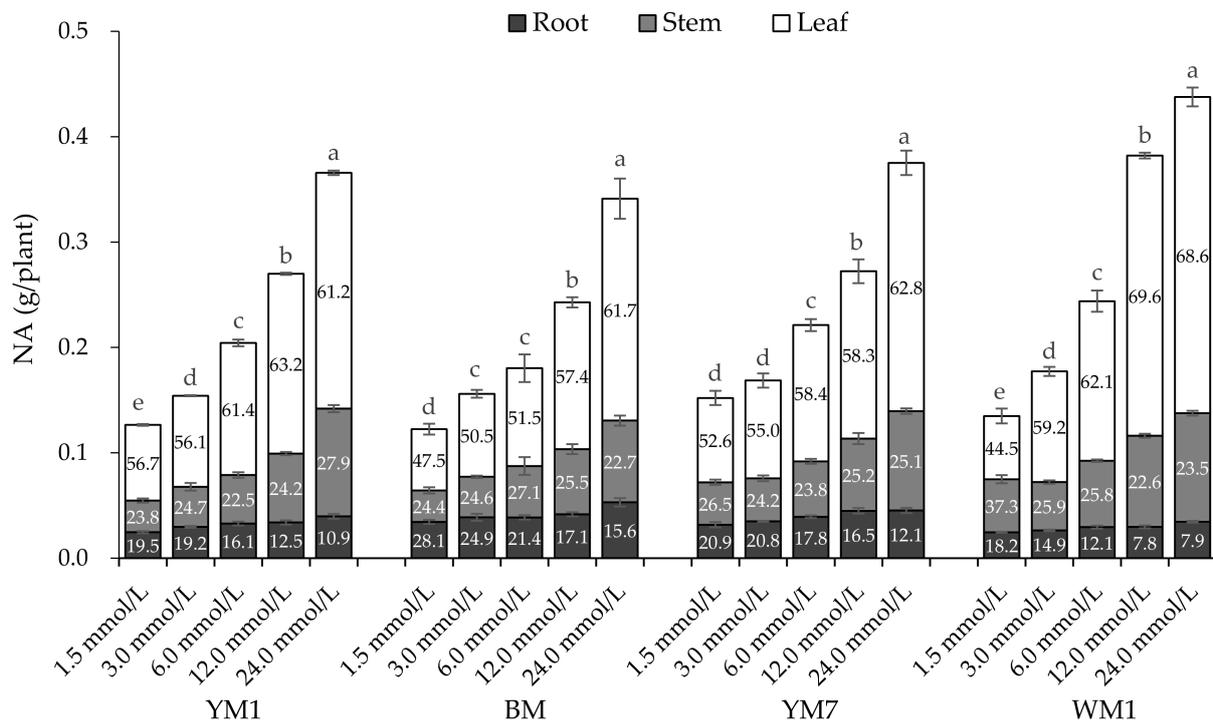


**Figure 1.** Effect of N treatments on hemp biomass weight. Different letters above the columns within a cultivar represent significant differences in total biomass at  $p = 0.05$ . Numbers within a column represent the percentage of root, stem, or leaf weight over the total biomass (%).

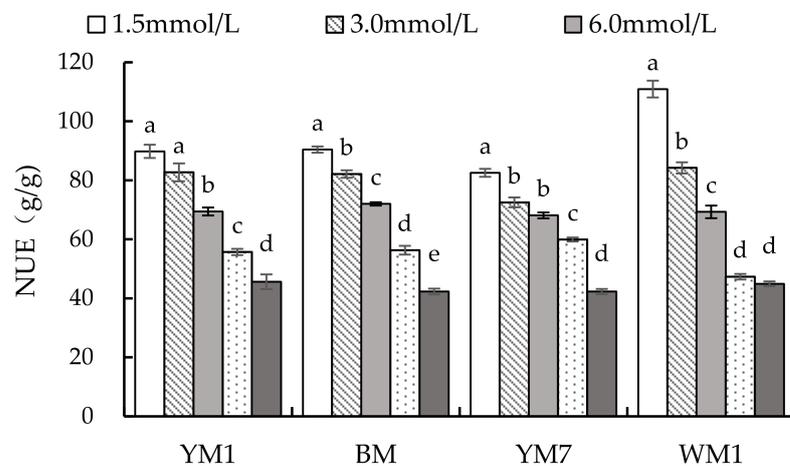
### 3.3. Effect of N Supply on NA and NUE in Hemp Plant

The nitrogen accumulation (NA) of hemp cultivars increased evidently with the increase in exogenous-N supply, reaching the maximum value at 24.0 mmol/L N level,

which was about three times that at the 1.5 mmol/L N level. Under all the N concentrations, NA in different plant parts was found as: leaf > stem > root, with the only exception for hemp cultivar BM under 1.5 and 3.0 mmol/L N levels. The ratio of NA in the leaves of BM and YM7 was increased with the increase in N concentration, while that of YM1 and WM1 increased first and then decreased, reaching the highest point at 12.0 mmol/L N level. The ratio of NA in the roots decreased with the increase in N concentration, and the ratio was reduced (about 50%) at 24.0 mmol/L N in comparison with that at 1.5 mmol/L N (Figure 2). However, the NUE of hemp cultivars was decreased significantly with the increase in exogenous-N supply and the lowest NUE value was observed at 24.0 mmol/L N that was about half as that at 1.5 mmol/L N (Figure 3). Among four hemp cultivars, WM1 performed well at 6.0 mmol/L N application.



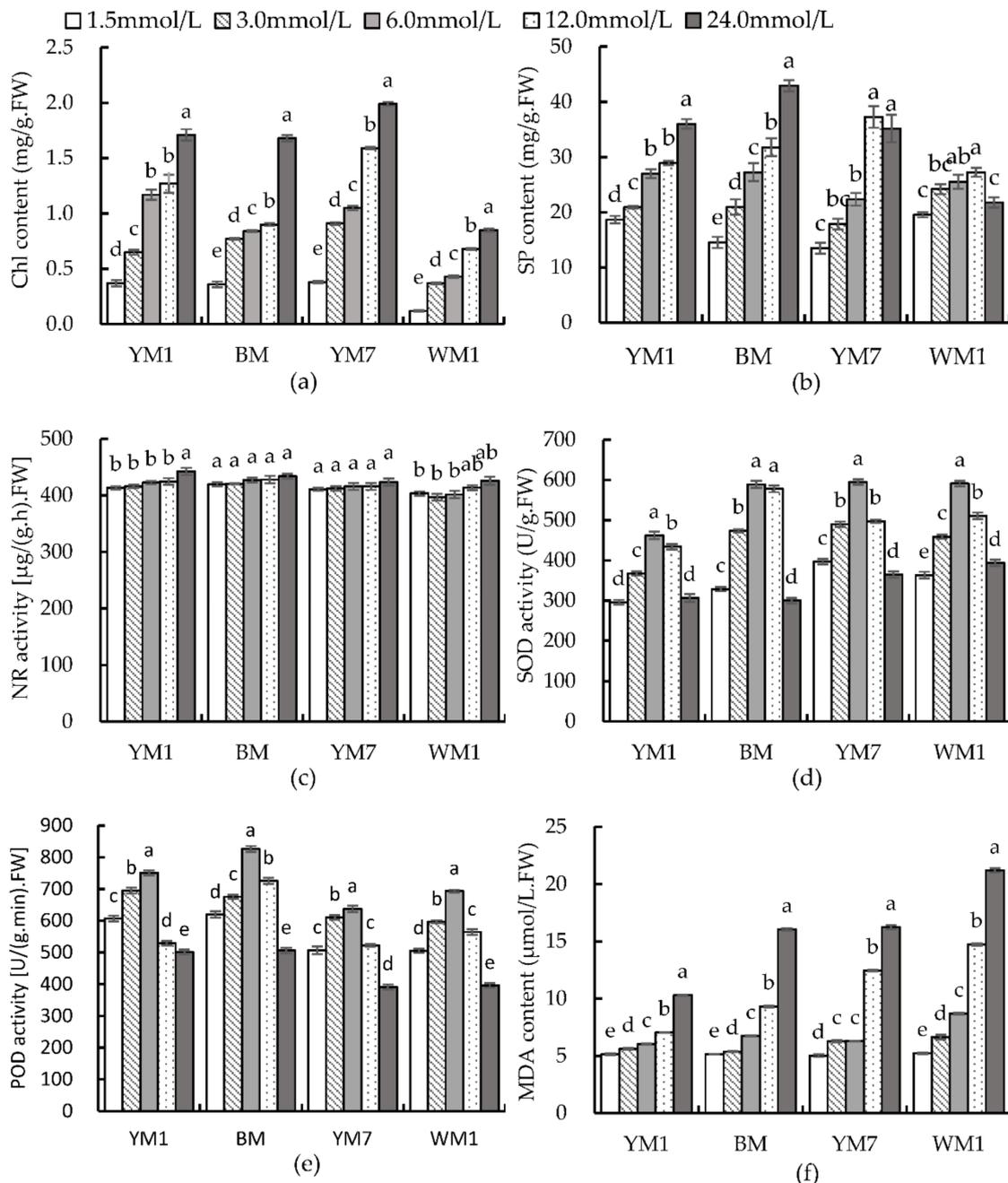
**Figure 2.** Effect of N treatments on N accumulation (NA) in hemp plant. Different letters above the columns within a cultivar represent significant differences in total N accumulation at  $p = 0.05$ . Numbers within the column represent the percentage of root, stem, or leaf N accumulation over the total N accumulation (%).



**Figure 3.** Effect of N treatments on N utilization efficiencies in hemp. Different letters above the columns within a cultivar represent significant differences in N utilization efficiencies at  $p = 0.05$ .

### 3.4. Effect of N Supply on Chl, SP, and NR in Hemp Leaf

Chlorophyll (Chl) content in hemp leaves were increased with the increase in exogenous-N supply, reaching maximum value at 24.0 mmol/L N, although not showing a linear correlation between Chl content and N level (Figure 4a).



**Figure 4.** Effect of N treatments on the physiological indicators of hemp leaf. Different letters above the columns within a cultivar represent significant differences in physiological indicators of leaf at  $p = 0.05$ . (a) The content of Chl of four hemp cultivars under different levels of N. (b) The content of SP of four hemp cultivars under different levels of N. (c) The activity of NR of four hemp cultivars under different levels of N. (d) The activity of SOD of four hemp cultivars under different levels of N. (e) The activity of POD of four hemp cultivars under different levels of N. (f) The content of MNA of four hemp cultivars under different levels of N.

The soluble protein (SP) contents of YM1 and BM were increased with the increase in exogenous-N supply, reaching a maximum value at 24.0 mmol/L N and significantly

surpassing those under other N concentrations; while the SP contents of YM7 and WM1 were increased first and then decreased, reaching the maximum value at 12.0 mmol/L (Figure 4b).

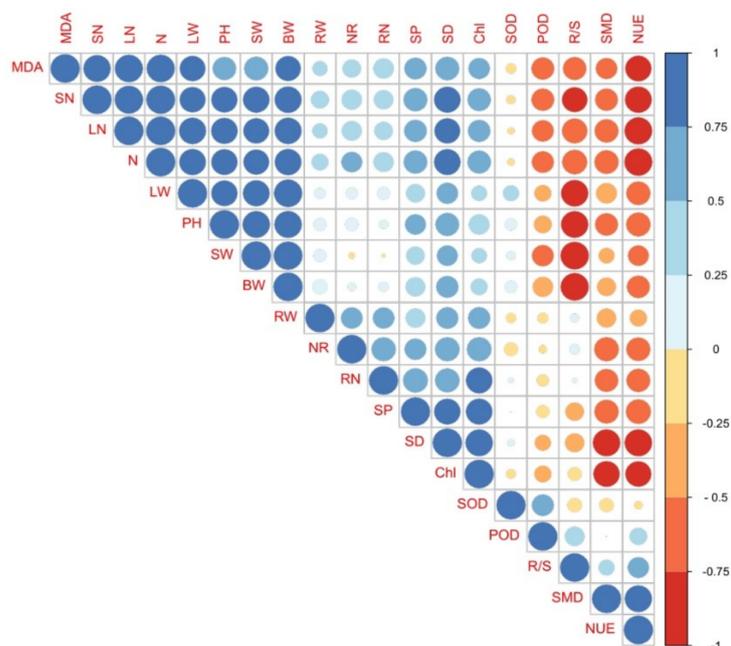
With the increase in exogenous-N supply, nitrate reductase (NR) activity in hemp leaves showed an increasing trend. Among the cultivars, NR activity in BM and YM7 did not change significantly under different N concentrations (Figure 4c).

### 3.5. Effect of N Supply on SOD, POD, and MDA in Hemp Leaf

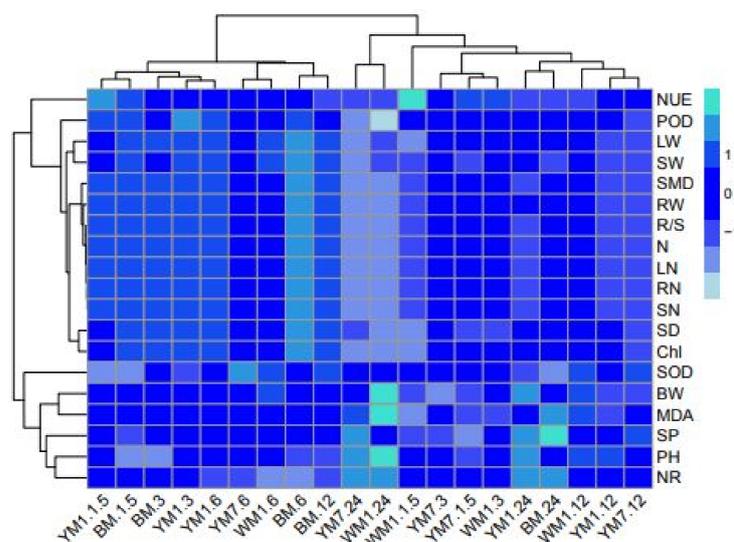
According to the results presented in Figure 4d,e, the activities of SOD and POD increased first and then decreased with the increase in exogenous-N supply, and they reached maximum value at 6.0 mmol/L N, which was significantly higher than those under other N concentrations (except for SOD in BM from 6.0 to 12.0 mmol/L N levels). In contrast, the malondialdehyde (MDA) content in hemp cultivars increased significantly with the increase in exogenous-N supply, while it showed a smaller increase from N levels 1.5 to 6.0 mmol/L and a larger increase from 6.0 to 24.0 mmol/L N level. From 1.5 to 6.0 mmol/L N level, the average MDA content of the four cultivars increased by 35% only, but it increased by 127% from 6.0 to 24.0 mmol/L N level (Figure 4f).

### 3.6. Relationships

In order to study the relationship between the studies' parameters, Pearson correlation was carried out (Figure 5). The results showed a significant positive relationship between plant height, stem diameter, leaf weight, stem weight, root weight, biomass weight, chlorophyll content, and N accumulation. Inversely, these attributes showed a negative relationship with SOD and POD activity. Furthermore, this relationship showed a close link between N accumulation and the growth of hemp plants. In addition, hierarchical clustering analysis showed a relation between interactive treatments and studied parameters (Figure 6).



**Figure 5.** Correlation between studied parameters of growth, antioxidant capacity, and N accumulation in hemp. PH, plant height; SD, stem diameter; LW, leaf weight; SW, stem weight; RW, root weight; BW, biomass weight; R/S, root to shoot ratio; SMD, stem mass density; Chl, chlorophyll content; SP, soluble protein; NR, nitrate reductase activity; SOD, superoxide dismutase; POD, peroxidase; MDA, malondialdehyde; RN, root nitrogen; SN, stem nitrogen; LN, leaf nitrogen; N, total nitrogen; NUE, nitrogen use efficiency.



**Figure 6.** A heatmap showing the relationship of treatments with the studied parameters of growth, antioxidant capacity, and N accumulation in hemp. The colors represent variations in the data. PH, plant height; SD, stem diameter; LW, leaf weight; SW, stem weight; RW, root weight; BW, biomass weight; R/S, root to shoot ratio; SMD, stem mass density; Chl, chlorophyll content; SP, soluble protein; NR, nitrate reductase activity; SOD, superoxide dismutase; POD, peroxidase; MDA, malondialdehyde; RN, root nitrogen; SN, stem nitrogen; LN, leaf nitrogen; N, total nitrogen; NUE, nitrogen use efficiency. YM1.1.5, cultivar YN1 + 1.5 mmol/L N; YM1.3, cultivar YN1 + 3 mmol/L N; YM1.6, cultivar YN1 + 6 mmol/L N; YM1.12, cultivar YN1 + 12 mmol/L N; YM1.24, cultivar YN1 + 24 mmol/L N; BM.1.5, cultivar BM + 1.5 mmol/L N; BM.3, cultivar BM + 3 mmol/L N; BM.6, cultivar BM + 6 mmol/L N; BM.12, cultivar BM + 12 mmol/L N; BM.24, cultivar BM + 24 mmol/L N; YM7.1.5, cultivar YM7 + 1.5 mmol/L N; YM7.3, cultivar YM7 + 3 mmol/L N; YM7.6, cultivar YM7 + 6 mmol/L N; YM7.12, cultivar YM7 + 12 mmol/L N; YM7.24, cultivar YM7 + 24 mmol/L N; WM1.1.5, cultivar WM1 + 1.5 mmol/L N; WM1.3, cultivar WM1 + 3 mmol/L N; WM1.6, cultivar WM1 + 6 mmol/L N; WM1.12, cultivar WM1 + 12 mmol/L N; WM1.24, cultivar WM1 + 24 mmol/L N.

#### 4. Discussion

Hemp is a short-day plant with a stronger stem and well-developed root system to avoid lodging and getting high yield [14,15]. Islam et al. [20] revealed that the dry weight per unit length of basal internodes was the main factor determining the mechanical strength of stems. However, Sperling et al. [21] found that high nitrogen (N) conditions limit photosynthetic productivity in almond trees, which is not good for dry matter accumulation. In the current study, the plant height and stem diameter of hemp were increased significantly with the increase in exogenous-N supply, but SMD was decreased significantly. Present results are consistent with the previous studies performed on rice [22,23]. There might be a reason that the plant height and stem diameter are sensitive to N, which lead toward insufficient dry matter accumulation during the rapid growth of hemp. We found that the average plant height and stem diameter of four cultivars were increased by 59.3% and 67.8% respectively, while the dry matter was increased by 32.8% only, when the exogenous-N supply was increased from 1.5 to 24.0 mmol/L. Our results are consistent with the previous study performed on wheat, where an excessive application of N fertilizer did not significantly increase the rate of dry matter accumulation [24].

In response to N deficiency in crops, assimilates are preferentially used for root development rather than shoot development [25]. Meanwhile, under a higher supply of N, the development of the aerial parts increased and the development of the roots decreased [26,27]. In the present study, the root/shoot dry weight ratio in hemp decreased significantly with the increase in exogenous-N supply, suggesting that excessive N application maximized the shoot development and minimized the root growth. These results are consistent with the previous studies on sweet pepper and maize crops [25,28]. Thus, we

observed that the weak plant stem and underdeveloped root were caused by excessive N application, which is prone to lodging and affects the yield in hemp.

Nitrogen application can improve the N content in soil, which is conducive to the absorption by crops [29]. After uptake, a large quantity of N is transferred to leaves, where it is assimilated into amino acids, proteins, and other nitrogenous compounds [30,31]. In the present study, increasing the supply of exogenous-N greatly increases the N accumulation in hemp plants. The accumulated N is mainly concentrated in leaves, and it corresponds to the Chl and SP of leaves significantly increasing, which is consistent with the research results of Li et al. on cotton [32]. Thus, a higher level of exogenous-N was beneficial to N accumulation and the synthesis of nitrogenous compounds in hemp. However, we found that the efficiency of accumulated N converted into dry matter in hemp was reduced at a higher level of exogenous-N, suggesting that the accumulated N was not effectively metabolized. Our results are consistent with a previous study on maize [33].

The activity of related metabolic enzymes is the key to improving the N utilization capacity of plants, such as NR; its activity is affected by the nitrate concentration in the plants [34,35]. The primary assimilation of N is accompanied by various enzymatic activities in higher plants. Nitrate reductase plays a central role in the transformation of  $\text{NO}_3^-$ ; thus, it regulates the  $\text{NO}_3^-$  and amino acids level in the plant cells. However, some investigations revealed that a small amount of nitrate is sufficient for enzymes induction; namely, the activity of NR is not induced by nitrate, when nitrate concentration was higher than a certain level [36]. The result of our experiment is consistent with the above report, because when the level of exogenous-N supply was 1.5 mmol/L, the accumulation of nitrate in leaves of hemp could induce NR to maintain a high activity, and further increasing the level of N supply had little effect on it. Thus, that the NR activity did not increase significantly is one of the major factors indicating that the accumulated N was not effectively metabolized under a high level of exogenous-N in hemp.

Reactive oxygen species (ROS) was considered to be toxic by-products of normal metabolism in plant, such as photosynthesis and respiration [37]. To control the level of ROS in cells, plants developed numerous strategies for the detoxification of ROS. Among antioxidative enzymes, SOD and POD play key roles in the ROS detoxification in cells [38,39]. In the present study, we observed that the levels of two antioxidant enzymes (SOD and POD) were decreased in the leaves of hemp when the level of exogenous-N supply was 12.0 mmol/L or more. These results are consistent with a previous study on wheat [40]. The application of N fertilizer beyond a tolerable limit may have adverse effects on plant growth; thus, it inhibited the activities of ROS scavenging enzymes, which resulted in increased oxidative stress.

Some evidence suggests that the excessive accumulation of ROS can cause a series of oxidative damages to proteins, lipids, and DNA, resulting in lipid peroxidation, cellular damage, and cell death [41–43]. The level of MDA is used normally to indicate the extent of lipid peroxidation in leaves. In the present study, the damage degree of membrane lipid peroxidation was increased significantly, when the level of exogenous-N supply was 12.0 mmol/L or more. Thus, our results indicated that hemp plants suffered from a greater degree of oxidative damage when the exogenous-N supply level exceeded 6.0 mmol/L, which might affect the normal physiological metabolism in hemp. The SP content of YM7 and WM1 decreased at 24 mmol/L N supplying level. These results have also been found in macrophytes, where excess N supply led to the reduction of osmotic regulation substance content, such as SP, reducing its stress resistance [44]. This might be linked to the oxidative stress in hemp, but further research is needed.

In addition, it is also worth paying attention to the accumulation of N far beyond its utilization capacity during the vegetative growth period when N is sufficient. One of the possible explanations might be that excess N is a reserve for later reproductive growth, which is a peak period of nutrient consumption in hemp [10,12]. It is important for hemp to adapt to a changeable variable environment. When N is sufficient, hemp can accumulate N as much as possible, and the stored N can be used for reproductive growth to alleviate the

adverse effects of late N deficiency in the environment. However, these contents need to be further studied in hemp, especially the situation of N absorption and utilization during the reproductive growth period.

## 5. Conclusions

In this study, an increase in the exogenous-N supplying level was good for hemp growth, but it was not to exceed 6.0 mmol/L during vegetative growth periods. Under excessive N supply, plants grow rapidly, but the dry matter accumulation was evidently insufficient, which weakened the hemp plants. Moreover, the large amount of N accumulated in plants could not be effectively assimilated and utilized, and it caused oxidative stress to hemp plants. Thus, the present study suggests that N application up to 6.0 mmol/L is sufficient to regulate the morpho-physiological attributes, antioxidant capacity, and N accumulation to achieve the optimal growth of hemp. Among four hemp cultivars, “Wanma 1” performed well at 6.0 mmol/L N application.

**Author Contributions:** Conceptualization, F.L.; methodology, W.Z.; formal analysis, Y.Y.; investigation, W.Z.; resources, F.L.; data curation, Y.Y. and G.D. (Gang Deng); writing—original draft preparation, Y.Y.; writing—review and editing, K.T. and G.D. (Guanghui Du); funding acquisition, F.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** The study was supported by China Agriculture Research System of MOF and MARA for Bast and Leaf Fiber Plants (CARS-16-E15).

**Data Availability Statement:** The data sets generated for this study are available on request to the corresponding author.

**Acknowledgments:** The authors gratefully acknowledge all staff members and students involved in the study.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Liu, Q.; Wu, K.; Fu, X.; Li, S.; Liu, X.; Gao, X. Sustainable crop yields from the coordinated modulation of plant growth and nitrogen metabolism. *Chin. Sci. Bull.* **2019**, *64*, 2633–2640. [CrossRef]
2. Asif, I.; Dong, Q.; Wang, Z.; Wang, X.G.; Gui, H.P.; Zhang, H.H.; Pang, N.C.; Zhang, X.L.; Song, M.Z. Growth and nitrogen metabolism are associated with nitrogen-use efficiency in cotton genotypes. *Plant Physiol. Biochem.* **2020**, *149*, 61–74. [CrossRef]
3. Ahmed, M.; Rauf, M.; Mukhtar, Z.; Saeed, N.A. Excessive use of nitrogenous fertilizers: An unawareness causing serious threats to environment and human health. *Environ. Sci. Pollut. Res.* **2017**, *24*, 26983–26987. [CrossRef]
4. Harm, V.B.; Jake, M.S.; Atina, G.C.; Carling, M.T.; Andrew, G.S.; Timothy, R.H.; Jonathan, E.P. The Draft Genome and Transcriptome of *Cannabis sativa*. *Genome Biol.* **2011**, *12*, R102. Available online: <http://genomebiology.com/2011/12/10/R102> (accessed on 12 November 2021).
5. Farinon, B.; Molinari, R.; Costantini, L.; Merendino, N. The seed of industrial hemp (*Cannabis sativa* L.): Nutritional Quality and Potential Functionality for Human Health and Nutrition. *Nutrients* **2020**, *12*, 1935. [CrossRef]
6. Ranalli, P.; Venturi, G. Hemp as a raw material for industrial applications. *Euphytica* **2004**, *140*, 1–6. [CrossRef]
7. Salentijn, E.M.J.; Petit, J.; Trindade, L.M. The Complex Interactions Between Flowering Behavior and Fiber Quality in Hemp. *Front. Plant Sci.* **2019**, *10*, 614. [CrossRef]
8. Liu, F.H.; Du, G.H.; Yang, Y.; Deng, G.; Tang, K.L. *Green and Efficient Cultivation Techniques for Industrial Hemp of Flower Heads and Leaf Uses*, 1st ed.; Yunnan University Press: Kunming, China, 2020; pp. 15–18.
9. Deng, G.; Du, G.; Yang, Y.; Bao, Y.; Liu, F. Planting Density and Fertilization Evidently Influence the Fiber Yield of Hemp (*Cannabis sativa* L.). *Agronomy* **2019**, *9*, 368. [CrossRef]
10. Kakabouki, I.; Kousta, A.; Folina, A.; Karydogianni, S.; Zisi, C.; Kouneli, V.; Papastylianou, P. Effect of Fertilization with Urea and Inhibitors on Growth, Yield and CBD Concentration of Hemp (*Cannabis sativa* L.). *Sustainability* **2021**, *13*, 2157. [CrossRef]
11. Forrest, C.; Young, J.P. The Effects of Organic and Inorganic Nitrogen Fertilizer on the Morphology and Anatomy of *Cannabis sativa* “Fédrina” (Industrial Fibre Hemp) Grown in Northern British Columbia, Canada. *J. Ind. Hemp* **2006**, *11*, 3–24. [CrossRef]
12. Papastylianou, P.; Kakabouki, I.; Travlos, I. Effect of Nitrogen Fertilization on Growth and Yield of Industrial Hemp (*Cannabis sativa* L.). *Not. Bot. Hort. Agrobot. Cluj-Napoca* **2017**, *46*, 197–201. [CrossRef]
13. Aubin, M.; Seguin, P.; Vanasse, A.; Tremblay, G.F.; Mustafa, A.F.; Charron, J. Industrial Hemp Response to Nitrogen, Phosphorus, and Potassium Fertilization. *CFTM* **2015**, *1*, 1–10. [CrossRef]

14. Sausserde, R.; Adamovics, A. Effect of nitrogen fertilizer rates on industrial hemp (*Cannabis sativa* L.) biomass production. In Proceedings of the International Multidisciplinary Scientific Geo Conference, Varna, Bulgaria, 16–22 June 2013; Volume 1, pp. 339–346.
15. Amaducci, S.; Errani, M.; Venturi, G. Response of hemp to plant population and nitrogen fertilization. *Ital. J. Agron.* **2002**, *6*, 103–111.
16. Wylie, S.E.; Ristvey, A.G.; Fiorellino, N.M. Fertility management for industrial hemp production: Current knowledge and future research needs. *GCB Bioenergy* **2021**, *13*, 517–524. [[CrossRef](#)]
17. Li, B.; Xin, W.; Sun, S.; Shen, Q.; Xu, G. Physiological and Molecular Responses of Nitrogen-starved Rice Plants to Re-supply of Different Nitrogen Sources. *Plant Soil* **2006**, *287*, 145–159. [[CrossRef](#)]
18. Wang, X.K. *Experimental Principle and Technology of Plant Physiology and Biochemistry*, 3rd ed.; Higher Education Press: Beijing, China, 2015; pp. 50–105.
19. Silveira, J.; Matos, J.; Cecatto, V.; Viegas, R.; Oliveira, J. Nitrate reductase activity, distribution, and response to nitrate in two contrasting Phaseolus species inoculated with Rhizobium spp. *Environ. Exp. Bot.* **2001**, *46*, 37–46. [[CrossRef](#)]
20. Islam, M.S.; Peng, S.; Visperas, R.M.; Ereful, N.; Bhuiya, M.S.U.; Julfiquar, A. Lodging-related morphological traits of hybrid rice in a tropical irrigated ecosystem. *Field Crop. Res.* **2007**, *101*, 240–248. [[CrossRef](#)]
21. Sperling, O.; Karunakaran, R.; Erel, R.; Yasuor, H.; Klipcan, L.; Yermiyahu, U. Excessive nitrogen impairs hydraulics, limits photosynthesis, and alters the metabolic composition of almond trees. *Plant Physiol. Biochem.* **2019**, *143*, 265–274. [[CrossRef](#)]
22. Wu, X.R.; Zhang, W.J.; Wu, L.M.; Wang, F.; Li, G.H.; Liu, Z.H.; Tang, S.; Ding, C.G.; Wang, S.H.; Ding, Y.F. Characteristics of lodging resistance of super-Hybrid indica rice and its response to nitrogen. *Sci. Agric. Sin.* **2015**, *48*, 2705–2717. [[CrossRef](#)]
23. Zhang, J.; Li, G.-H.; Song, Y.-P.; Zhang, W.-J.; Yang, C.-D.; Wang, S.-H.; Ding, Y.-F. Lodging Resistance of Super-Hybrid Rice Y Liangyou 2 in Two Ecological Regions. *Acta Agron. Sin.* **2013**, *39*, 682. [[CrossRef](#)]
24. Song, M.D.; Li, Z.P.; Feng, H. Effects of irrigation and nitrogen regimes on dry matter dynamic accumulation and yield of winter wheat. *Trans. Chin. Soc. Agric. Eng.* **2016**, *32*, 119–126. [[CrossRef](#)]
25. Grasso, R.; de Souza, R.; Peña-Fleitas, M.T.; Gallardo, M.; Thompson, R.B.; Padilla, F.M. Root and crop responses of sweet pepper (*Capsicum annuum*) to increasing N fertilization. *Sci. Hortic.* **2020**, *273*, 109645. [[CrossRef](#)]
26. Garnett, T.; Conn, V.; Kaiser, B.N. Root based approaches to improving nitrogen use efficiency in plants. *Plant Cell Environ.* **2009**, *32*, 1272–1283. [[CrossRef](#)]
27. Drew, M.C.; Saker, L.R.; Ashley, T.W. Nutrient Supply and the Growth of the Seminal Root System in Barley. *J. Exp. Bot.* **1973**, *24*, 1189–1202. [[CrossRef](#)]
28. Anderson, E.L. Tillage and N fertilization effects on maize root growth and root:shoot ratio. *Plant Soil* **1988**, *108*, 245–251. [[CrossRef](#)]
29. Ju, X.T.; Gu, B.J. Status-quo, problem and trend of nitrogen fertilization in China. *J. Plant Nutr. Fertil.* **2014**, *20*, 783–795. [[CrossRef](#)]
30. Qu, L.B.; Gu, H.Y.; Liu, J.J.; Qing, G.J. *Plant Biology, 1st ed*; The Science Press: Beijing, China, 2012; pp. 303–320.
31. Ren, B.; Dong, S.; Zhao, B.; Liu, P.; Zhang, J. Responses of Nitrogen Metabolism, Uptake and Translocation of Maize to Waterlogging at Different Growth Stages. *Front. Plant Sci.* **2017**, *8*, 1216. [[CrossRef](#)] [[PubMed](#)]
32. Li, C.P.; Dong, H.L.; Liu, A.Z.; Liu, J.R.; Li, R.Y.; Sun, M.; Li, Y.B.; Mao, S.C. Effects of nitrogen application rates on physiological characteristics of functional leaves, nitrogen use efficiency and yield of cotton. *J. Plant Nutr. Fertil.* **2015**, *21*, 81–91. [[CrossRef](#)]
33. Jian, L.L.; Han, L.S.; Han, X.R.; Zhan, X.M.; Zuo, R.H.; Wu, Z.C.; Yuan, C. Effects of nitrogen on growth, root morphological traits, nitrogen uptake and utilization efficiency of maize seedlings. *J. Plant Nutr. Fertil.* **2011**, *17*, 247–253. [[CrossRef](#)]
34. Iqbal, A.; Dong, Q.; Wang, X.; Gui, H.; Zhang, H.; Zhang, X.; Song, M. Variations in Nitrogen Metabolism are Closely Linked with Nitrogen Uptake and Utilization Efficiency in Cotton Genotypes under Various Nitrogen Supplies. *Plants* **2020**, *9*, 250. [[CrossRef](#)] [[PubMed](#)]
35. Krapp, A.; Berthomé, R.; Orsel, M.; Mercey-Boutet, S.; Yu, A.; Castaigns, L.; Elftieh, S.; Major, H.; Renou, J.-P.; Daniel-Vedele, F. Arabidopsis Roots and Shoots Show Distinct Temporal Adaptation Patterns toward Nitrogen Starvation. *Plant Physiol.* **2011**, *157*, 1255–1282. [[CrossRef](#)] [[PubMed](#)]
36. Chen, B.-M.; Wang, Z.-H.; Li, S.-X.; Wang, G.-X.; Song, H.-X.; Wang, X.-N. Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. *Plant Sci.* **2004**, *167*, 635–643. [[CrossRef](#)]
37. Mittler, R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **2002**, *7*, 405–410. [[CrossRef](#)]
38. Bowler, C.; Montagu, M.V.; Inze, D. Superoxide Dismutase and Stress Tolerance. *Annu. Rev. Plant Biol.* **1992**, *43*, 83–116. [[CrossRef](#)]
39. Willekens, H.; Chamnongpol, S.; Davey, M.; Schraudner, M.; Langebartels, C.; Van Montagu, M.; Inzé, D.; Van Camp, W. Catalase is a sink for H<sub>2</sub>O<sub>2</sub> and is indispensable for stress defence in C<sub>3</sub> plants. *EMBO J.* **1997**, *16*, 4806–4816. [[CrossRef](#)] [[PubMed](#)]
40. Kong, L.; Xie, Y.; Hu, L.; Si, J.; Wang, Z. Excessive nitrogen application dampens antioxidant capacity and grain filling in wheat as revealed by metabolic and physiological analyses. *Sci. Rep.* **2017**, *7*, 43363. [[CrossRef](#)]
41. Mitsuhara, I.; Malik, K.A.; Miura, M.; Ohashi, Y. Animal cell-death suppressors Bcl-xL and Ced-9 inhibit cell death in tobacco plants. *Curr. Biol.* **1999**, *9*, 775–778. [[CrossRef](#)]
42. Ishida, H.; Anzawa, D.; Kokubun, N.; Makino, A.; Mae, T. Direct evidence for non-enzymatic fragmentation of chloroplastic glutamine synthetase by a reactive oxygen species. *Plant Cell Environ.* **2002**, *25*, 625–631. [[CrossRef](#)]

- 
43. Domínguez-Valdivia, M.D.; Aparicio-Tejo, P.M.; Lamsfus, C.; Cruz, C.; Martins-Loução, M.A.; Moran, J.F. Nitrogen nutrition and antioxidant metabolism in ammonium-tolerant and -sensitive plants. *Physiol. Plant.* **2008**, *132*, 359–369. [[CrossRef](#)]
  44. Liu, H.W.; Zhang, R.R.; Liu, Y.L.; Xie, C.H.; Lin, H.; Lin, Z.X.; Ye, W.H. Effect of Nitrogen on Pennisetum sp. Seeding Stage Growth and Photosynthetic Physiological Characteristic. *Northern Hortic.* 2016, pp. 133–138. Available online: <http://bfyy.paperonce.org/oa/DArticle.aspx?type=view&id=201608037> (accessed on 12 November 2021).