agronomy

MDPI

Article

Nitrogen Use Efficiency of Watermelon Grafted onto 10 Wild Watermelon Rootstocks under Low Nitrogen Conditions

Muhammad Azher Nawaz [1,2,†], Xiaojie Han [1,†], Chen Chen [1], Zuhua Zheng [1], Fareeha Shireen [1], Zhilong Bie [1] and Yuan Huang [1,*]

1. Key Laboratory of Horticultural Plant Biology, Ministry of Education/College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan 430070, China; azher.nawaz@uos.edu.pk (M.A.N.); hanxiaojie@163.com (X.H.); cuiyanhuashi@163.com (C.C.); zuhzheng@163.com (Z.Z.); fareehamalik63@yahoo.com (F.S.); biezl@mail.hzau.edu.cn (Z.B.)
2. Department of Horticulture, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan

* Correspondence: huangyuan@mail.hzau.edu.cn; Tel.: +86-27-87282010

† These authors contributed equally to this work.

Received: 3 October 2018; Accepted: 5 November 2018; Published: 12 November 2018

check for updates

Abstract: Nitrogen availability is the key determinant of plant growth and development. The improvement of nitrogen use efficiency (NUE) in crops is an important consideration. In fruit and vegetables, such as watermelon, rootstocks are often utilized to control soil borne diseases and improve plant performance to a range of abiotic stresses. In this study, we evaluated the efficacy of 10 wild watermelon rootstocks (ZXG-516, ZXG-941, ZXG-945, ZXG-1250, ZXG-1251, ZXG-1558, ZXG-944, ZXG-1469, ZXG-1463, and ZXG-952) to improve the plant growth and nitrogen use efficiency (NUE) of the watermelon cultivar: Zaojia 8424. Nitrogen use efficiency (NUE) is a comprehensive parameter that represents the ability of a plant to absorb nitrogen (N) and convert the supplied resources to the dry biomass. Wild watermelon rootstocks substantially improved plant growth, rate of photosynthesis, stomatal conductivity, intercellular carbon dioxide concentration, rate of transpiration, nitrogen uptake efficiency, nitrogen use efficiency, and nitrogen utilization efficiency of watermelon. NUE of watermelon grafted onto ZXG-945, ZXG-1250, and ZXG-941 was improved by up to 67%, 77%, and 168%, respectively, at optimum N supply. Similarly, at low N supply (0.2 mM), NUE of watermelon grafted onto ZXG-1558 and ZXG-516 was improved by up to 104% and 175%, respectively. In conclusion, grafting onto some wild rootstocks can improve nitrogen use efficiency of watermelon, and this improved nitrogen use efficiency could be attributed to better N uptake efficiency of wild watermelon rootstocks.

Keywords: vegetable grafting; *Citrullus lanatus*; wild watermelon; nitrogen use efficiency; ion uptake

1. Introduction

Nitrogen (N) is a key nutrient required by plants for their growth and development. N is a component of amino acids, nucleic acid, proteins, chlorophyll, and hormones [1]. The availability of N positively influences the plant's architecture, photosynthesis and photosynthates allocation, flowering, and fruit development [2–4]. Roots absorb N in the form of nitrate (NO_3^-) and ammonium (NH_4^+) from the medium. Nitrate and ammonium transporters are involved in the uptake of NO_3^- and NH_4^+. Usually the concentration of NH_4^+ in the xylem sap is low compared with NO_3^- ions. Roots transport these ions to the aboveground plant parts (shoot). During N assimilation, NO_3^- is converted to NH_4^+ through cytosolic nitrate reductase. This NH_4^+ is then converted into glutamine or glutamate. The compounds that are synthesized in this process are utilized as a precursor in the formation of amino acids, proteins, and other N-containing metabolites [4,5]. N-containing compounds play a central role in the growth and development of plants.

Watermelon is commercially cultivated on a large scale worldwide. China is the main producer and contributes to 67.5% of the World's watermelon produce. A large amount of nitrogenous fertilizers are required for optimum plant production. According to the estimates of the National Bureau of Statistics of China, 24 million tons of nitrogen-containing fertilizers are utilized annually [6]. N-containing fertilizers are applied to fulfill the plant requirements; however, only 30% to 40% of the fertilizer is utilized by the crops. The remaining amount of fertilizer is wasted by means of leaching and volatilization, resulting in loss of resources and environmental pollution [7]. Nutrient use efficiency is generally referred to as the measurement of a plant's capacity to absorb and use nutrients to produce biomass (i.e., harvestable yield). The variation of nitrogen use efficiency (NUE) among different plant species and varieties is because of uptake efficiency or use efficiency [8]. For nitrogen, it is measured on the basis of plant dry weight per unit N supplied (g dry weight (DW)/g N). This parameter is calculated based on plant N uptake and use capacity [9].

Several approaches are used to enhance the ion uptake efficiency of the crops. For instance, breeding for nutrient efficient cultivars, root architecture modifications [10–12], use of efficient methods for fertilizer application, and soil microbes are employed to obtain maximum crop yields with less fertilizer use [13,14]. In fruit and vegetable crops, rootstocks are utilized to improve nutrient use efficiency of elite scion cultivars [13–16]. NUE of grafted transplants is dependent on the genetic potential of the scion and the rootstock, and their interaction. Rootstocks improve the ion uptake in several plant species [4,16–22]. Selection of an appropriate rootstock for a specific scion cultivar is important in order to enhance water and ion absorption, nutrient use efficiency, and plant growth and yield [23,24]. Currently, the exploration and development of ion-specific rootstock [25] and specific ion-efficient rootstock is gaining the attention of plant biologists [14,23,24].

Wild watermelon genotypes can be an important rootstock for this plant species because they have good grafting compatibility and have no negative impact on fruit quality compared with other rootstocks, such as some cultivars of bottle gourd and pumpkin [14]. However, according to the best of our knowledge, the ability of wild watermelon rootstocks to improve the NUE of watermelon is not investigated. Thus, we evaluated the effect of 10 watermelon genotypes on plant growth, relative chlorophyll content, photosynthetic assimilation, nitrogen uptake efficiency, nitrogen utilization efficiency, and NUE under low and optimum levels of N supply.

2. Materials and Methods

2.1. Rootstocks and Scion Utilized, and Experimental Details

The experiments were conducted in a growth chamber at Huazhong Agricultural University, China. The watermelon cultivar "Zaojia 8424 (ZJ)", developed by Xinjiang Academy of Agricultural Sciences, China, and 10 wild watermelon genotypes (ZXG-516, ZXG-941, ZXG-945, ZXG-1250, ZXG-1251, ZXG-1558, ZXG-944, ZXG-1469, ZXG-1463, and ZXG-952) were used as plant materials. Seeds were planted in plugged trays filled with a mixture of peat, perlite, and vermiculite. Rootstock seeds were sown five days earlier than the scion seeds. A total of 11 grafting combinations were produced that included ZJ self-grafted (ZJ/ZJ) and ZJ grafted onto 10 wild watermelon genotypes. For the preparation of grafted watermelon plants, the hole-insertion grafting method was used. The true leaves and growing point of the rootstock were carefully and completely removed just above the cotyledonary leaves, and a slanting hole was made with a wooden or plastic gimlet. The hypocotyl portion of the scion was prepared by making a slanting cut, with a tapering end for easy insertion [15,26]. During the healing period, plants were covered with a plastic sheet to maintain high humidity. The plastic sheet was removed for 30 min in the morning and afternoon, each day, to avoid the excessive increase of humidity. The film was completely removed after seven days of grafting. During this period, no nutrient solution was used and plants were simply irrigated with tap water until shifting to the nutrient solution. In this study, the self-grafted plants, of watermelon cultivar ZJ, were considered as the control. After healing and successful graft-union formation, uniform plants were shifted to plastic containers (8 L) filled with nutrient solution. In each container nine plants were shifted. In this study, two concentrations of N (0.2 mM and 9 mM) were used. The nutrient solution contained 3.5 Mm of $Ca(NO_3)_2$, 6 Mm of KCl, 0.5 mM of $Ca(H_2PO_4)_2$, 1 mM of $MgSO_4$, 1 mM of $Mg(NO_3)_2$, 89.7 µM of Na_2Fe-EDTA, 46.3 µM of H_3BO_3, 9.5 µM of $MnSO_4$, 0.8 µM of $ZnSO_4$, 0.3 µM of $CuSO_4$, and 0.1 µM of $(NH_4)_2MoO_4$. The low N nutrient solution was prepared by using 0.1 mM of $Ca(NO_3)_2$, no $Mg(NO_3)_2$, and all other nutrients were kept similar [4]. In this study, 11 grafting combinations and two levels of nitrogen were used. All the treatments were repeated three times. Each replicate contained nine grafted watermelon plants. During the experimental period, the nutrient solution was regularly changed after every five days to evade the deficiency of a specific ion. Aeration was provided to the roots by an air pump working every 2 h during the experimental period. The temperature of the growth chamber was adjusted to 28 and 18 °C (day/night), respectively, and the relative humidity was maintained at 65%. Plants were harvested for growth and ionic analysis after 20 days of N application.

2.2. Plant Growth

Plant growth was measured by using three uniform plants from each replication. All the plants were divided into root, rootstock stem, scion stem, and leaf, and packed in paper bags. These samples were dried in an oven at 105 °C for a short time (15 min), and then the temperature was reduced to 72 °C for three days. The dry weight was measured using an electric balance.

2.3. Relative Chlorophyll Content (SPAD Index) and Leaf Photosynthetic Assessment

To measure the SPAD index, a fully developed leaf (third leaf from the top) was used. Photosynthesis related parameters, such as photosynthetic rate, stomatal conductance, intercellular CO_2, and transpiration rate, were measured using the Li-6400XT, LI-COR, Lincoln, NE, USA. The chamber was adjusted to 25 °C (temperature), 360 µM/mol (CO_2), and 800 µM/m^2/s (photosynthetic photon-flux density).

2.4. Nitrogen Estimation and Nitrogen Use Efficiency

The dried plant samples were ground to a powder and digested with H_2SO_4–H_2O_2 (*v/v*, 5:1). N content was measured using the Kjeldahl method described by [27]. To measure the NUE, the following formulas were used [4,28]:

$$\text{Nitrogen uptake efficiency} = \frac{\text{N accumulated in plants}}{\text{N supplied}}\ (\text{mg N/mg N}) \tag{1}$$

$$\text{Nitrogen use efficiency} = \frac{\text{Dry weight of plant}}{\text{N supplied}}\ (\text{mg DW/mg N}) \tag{2}$$

$$\text{Nitrogen utilization efficiency} = \frac{\text{Dry weight of plant}}{\text{N accumulated in plants}}\ (\text{mg DW/mg N}) \tag{3}$$

2.5. Statistical Analysis

SPSS statistical software (IBM SPSS 22.0, IBM-Corporation, New York, USA) was used to perform the statistical analysis. The two-way analysis of variance was performed. Values were means ± SE of three replicates. *, **, and *** represent $p \leq 0.05$, 0.01, and 0.001, respectively, and ns means not significant.

3. Results

3.1. *Wild Watermelon Rootstocks Improve the Growth of Watermelon Scion*

The dry weights (DW) of the root, rootstock stem, scion stem, and the leaf of the watermelon plants were obviously affected by the use of different wild watermelon rootstocks, N availability, and their interaction (Figure 1). We observed that the dry weights of the root, rootstock stem, scion stem, and the leaf of the watermelon plants grown at the optimum level of N (9 mM) were obviously higher compared with the low level of N supply (0.2 mM), with a few exceptions. Considering the whole plant DW, at the optimum level of N (9 mM) watermelon plants grafted onto ZXG-941, ZXG-1250, ZXG-945, and ZXG-952 performed better compared with the watermelon grafted onto other rootstocks. However, at the low level of N supply (0.2 mM), watermelon plants grafted onto ZXG-516 and ZXG-1558 attained more DW compared with other rootstocks. Interestingly, the DW of watermelon plants grafted onto most of the wild watermelon rootstocks was higher compared with the self-grafted watermelon plants, and none of the rootstocks performed poorly compared with the self-grafted watermelon plants (Figure 1e).

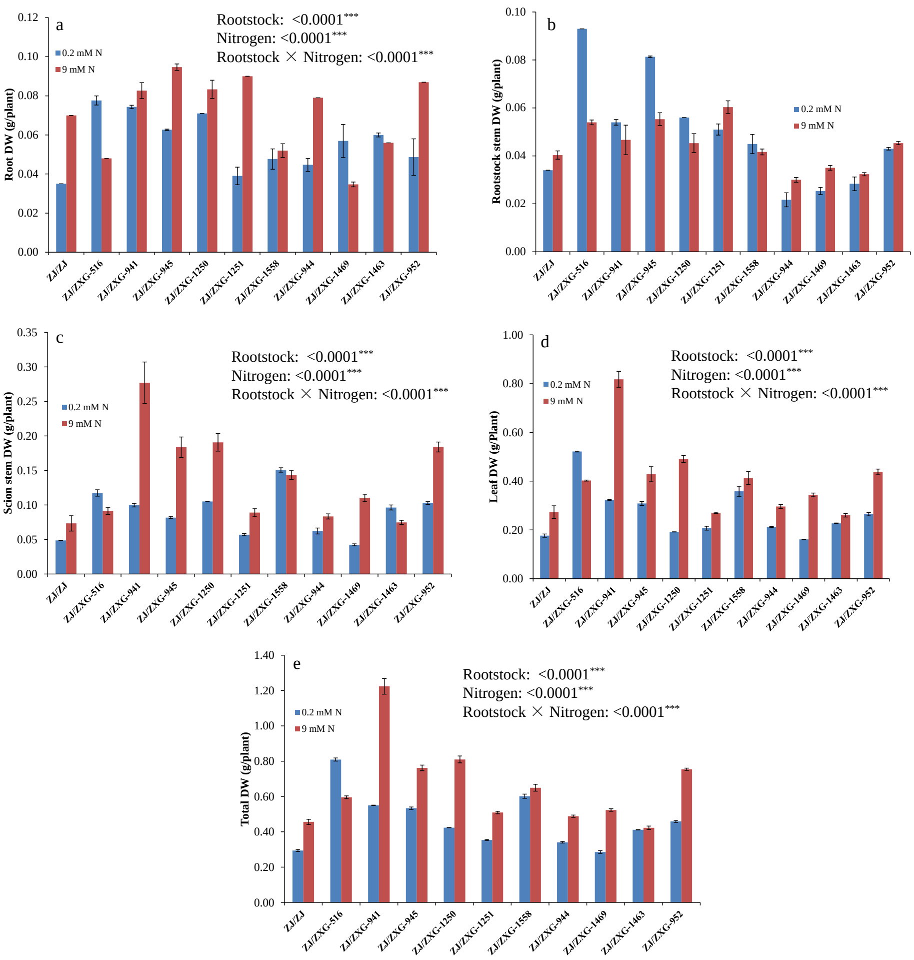

Figure 1. Root dry weight (**a**), rootstock stem dry weight (**b**), scion stem dry weight (**c**), leaf dry weight (**d**), and total dry weight (**e**) of watermelon cultivar Zaojia 8424 (ZJ), self-grafted (ZJ/ZJ) and grafted onto 10 different wild watermelon rootstocks, under normal (9 mM) and low (0.2 mM) nitrogen (N) conditions. Values are means ± standard error (SE) of three replicates. *** represent $P \leq 0.05$.

3.2. *Wild Watermelon Rootstocks Improve the Photosynthetic Responses of Watermelon Scion*

Photosynthetic assimilation, transpiration rate, water use efficiency, and SPAD index (relative chlorophyll content) of watermelon cultivar Zaojia 8424 (ZJ) was obviously affected by the N availability, rootstock–scion combinations, and their interaction (Figure 2a,d–f). Stomatal conductance and intercellular CO_2 were altered by the use of rootstock and the interaction between rootstock and N availability; however, these were not affected by N availability (Figure 2b,c). At low N (0.2 mM), the photosynthetic assimilation of watermelon grafted onto ZXG-1251, ZXG-1558, ZXG-944, and ZXG-1469 was substantially higher compared with the self-grafted watermelon plants. However, at the optimum level of N supply (9 mM N), the photosynthetic assimilation of watermelon grafted onto most of the rootstocks was improved, except for the watermelon grafted onto ZXG-1463 that was similar with the control (self-grafted ZJ). Some of the rootstock–scion combinations, such as watermelon grafted onto ZXG-1251, ZXG-1558, ZXG-944, and ZXG-1469, performed exceptionally well for the photosynthetic assimilation, and the difference between photosynthetic assimilation at low and optimum levels of N supply was negligible (Figure 2a). The stomatal conductance of watermelon grafted onto ZXG-1251 was substantially enhanced at the low level of N (0.2 mM); and stomatal conductance of watermelon grafted onto ZXG-945, ZXG-1250, ZXG-1251, and ZXG-1469 was improved at high N (9 mM) compared with the control (self-grafted ZJ). The use of several wild watermelon rootstocks improved the intercellular CO_2 and the transpiration rate of watermelon scion compared with the self-grafted watermelon at low and optimum levels of nitrogen supply (Figure 2c,d). Water use efficiency was also affected by the different levels of N, rootstock, and their interaction (Figure 2e).

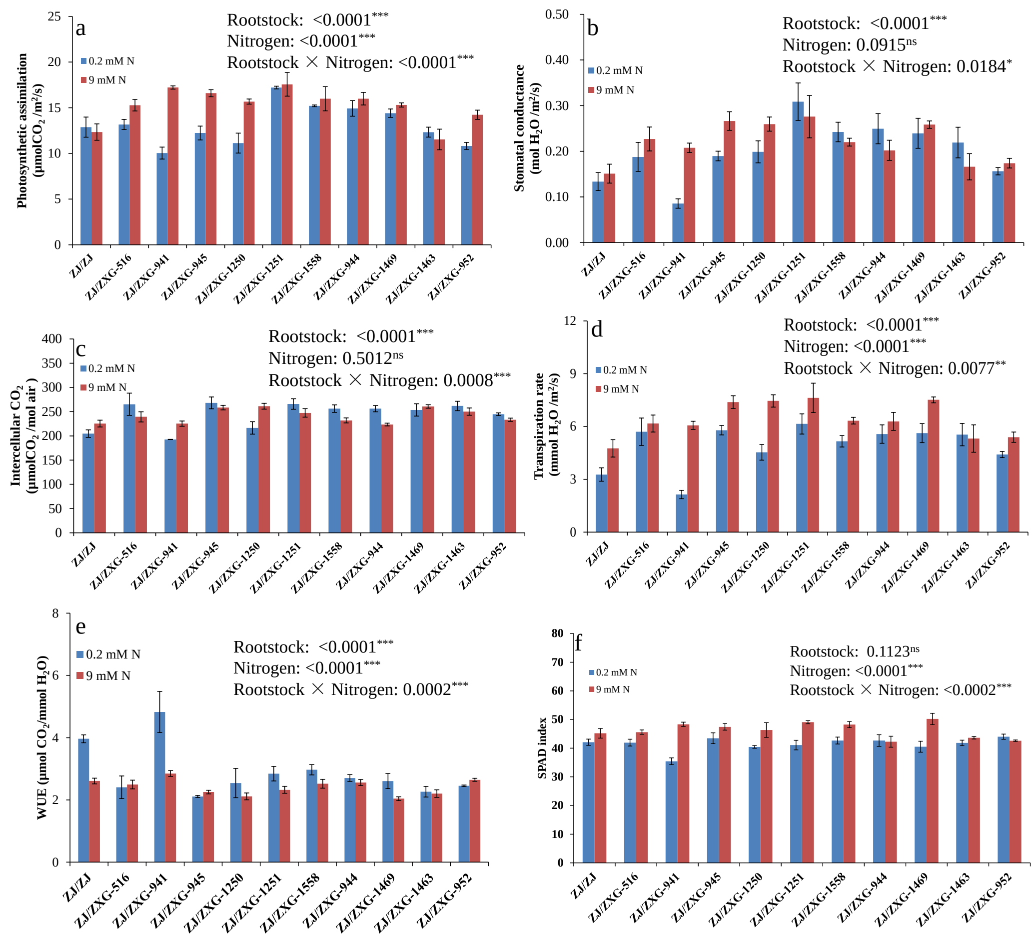

Figure 2. Photosynthetic assimilation (**a**), stomatal conductance (**b**), intercellular CO_2 (**c**), transpiration rate (**d**), water use efficiency (**e**), and SPAD index (relative chlorophyll content) (**f**) of watermelon cultivar Zaojia 8424 (ZJ), self-grafted (ZJ/ZJ) and grafted onto 10 different wild watermelon rootstocks under normal (9 mM) and low (0.2 mM) N conditions. Values are means ± SE of three replicates. *, **, and *** represent $P \leq 0.05$, 0.01 and 0.001, respectively, and ns means not significant.

3.3. Wild Watermelon Rootstocks Improve the NUE

N uptake efficiency, N utilization efficiency, and N use efficiency of grafted watermelon plants was affected by the rootstock combinations, N availability, and their interaction (Figure 3). At low N supply (0.2 mM), the N uptake efficiency of watermelon plants grafted onto ZXG-516, ZXG-945, ZXG-1250, ZXG-1558, and ZXG-952 was substantially improved compared with the self-grafted watermelon plants. Similarly, at the optimum level of N supply (9 mM), the N uptake efficiency of the watermelon plants grafted onto ZXG-516, ZXG-941, ZXG-945, ZXG-1250, ZXG-1558, ZXG-1469, and ZXG-952 was better compared with the self-grafted watermelon plants (Figure 3a). The N uptake efficiency of the watermelon plants grafted onto ZXG-516 was 2.68-fold compared with the self-grafted watermelon plants. This rootstock–scion combination "ZJ8424 grafted onto ZXG-516" was better compared with any other rootstock–scion combination evaluated in this study at the low N supply (Figure 3a).

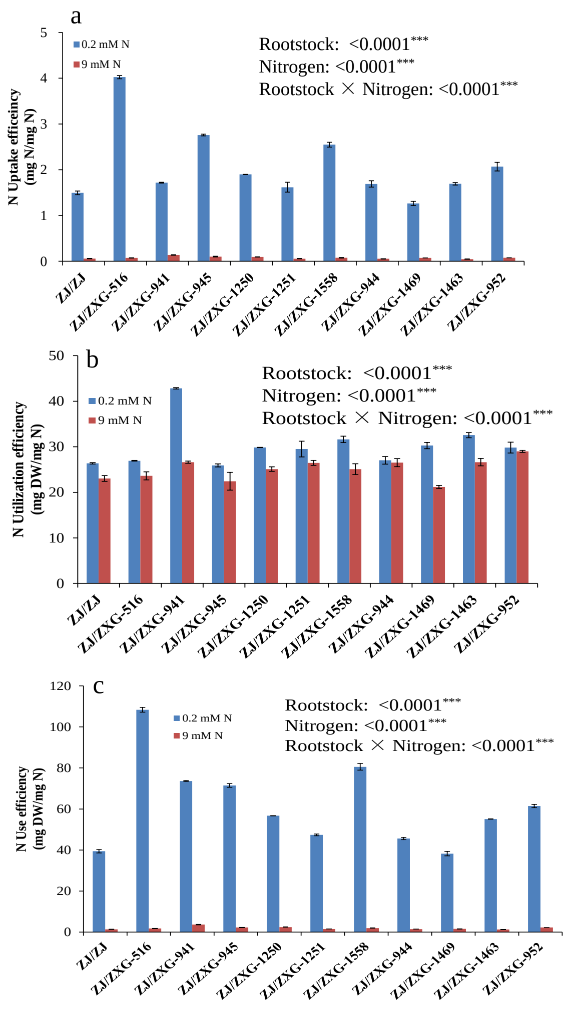

Figure 3. N uptake efficiency (**a**), N utilization efficiency (**b**), and N use efficiency (**c**) of watermelon cultivar Zaojia 8424 (ZJ), self-grafted (ZJ/ZJ) and grafted onto 10 different wild watermelon rootstocks under normal (9 mM) and low (0.2 mM) N conditions. Values are means ± SE of three replicates. *** represent $P \leq 0.05$.

The result for N utilization efficacy showed that the N utilization efficacy of watermelon grafted onto seven of wild watermelon rootstocks (ZXG-941, ZXG-1250, ZXG-1251, ZXG-1558, ZXG-1469, ZXG-1463, and ZXG-952) was improved compared with the self-grafted watermelon plants at the low N supply (0.2 mM). At the optimum N supply (9 mM), generally the N utilization efficacy was reduced compared with the low N supply (0.2 mM); however, the N utilization efficacy of watermelon plants grafted onto ZXG-952, ZXG-941, ZXG-1250, ZXG-944, and ZXG-1463 was better compared with the self-grafted watermelon plants. At low N (0.2 mM), the N utilization efficacy of watermelon plants grafted onto ZXG-941 was improved by 62% compared with the self-grafted watermelon plants (Figure 3b).

N use efficiency of watermelon grafted onto different wild watermelon rootstock was also apparently affected by the rootstock, N availability, and their interaction. The N use efficiency at low N was many folds higher compared with the optimum level of N supply (Figure 3c). At low N, the N use efficiency of watermelon plants grafted onto nine of wild watermelon rootstocks (ZXG-516, ZXG-941, ZXG-945, ZXG-1250, ZXG-1251, ZXG-1558, ZXG-944, ZXG-1463, and ZXG-952) was improved compared with the self-grafted watermelon plants, except ZXG-1469 that was similar with the control (self-grafted ZJ). Similarly, at the optimum N (9 mM), the N use efficiency of watermelon grafted onto six different wild watermelon rootstocks (ZXG-516, ZXG-941, ZXG-945, ZXG-1250, ZXG-1558, and ZXG-952) was substantially enhanced compared with the self-grafted watermelon. The N use efficiency of watermelon grafted onto ZXG-516 and ZXG-1558 was improved by 175% and 104%, respectively, compared with the self-grafted watermelon at the low N supply. Similarly, at optimum N (9 mM), the N use efficiency of watermelon grafted onto ZXG-941, ZXG-1250, and ZXG-945 was enhanced by up to 168%, 77%, and 67%, respectively, compared with the self-grafted watermelon.

3.4. Percentage Biomass and N Allocation in the Scion

The percentage biomass allocation and percentage N allocation in the scion was apparently affected by the rootstock, N availability, and their interaction (Figure 4). At low N availability (0.02 mM) the parentage biomass allocation in the scion was substantially higher by the use of ZXG-941, ZXG-1558, and ZXG-1559 rootstocks. The critical observation of Figure 4b shows that the N allocation to the scion, in the cases of these rootstocks (ZXG-941, ZXG-1558, and ZXG-1559), was also higher compared with the self-grafted watermelon plants (ZJ/ZJ).

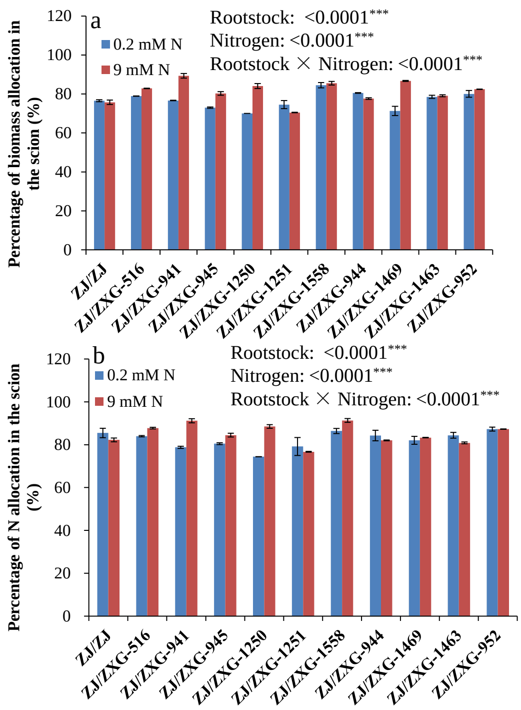

Figure 4. Percentage biomass allocation in the scion (**a**) and percentage of N allocation in the scion (**b**) of watermelon cultivar Zaojia 8424 (ZJ), self-grafted (ZJ/ZJ) and grafted onto 10 different wild watermelon rootstocks under normal (9 mM) and low (0.2 mM) N conditions. Values are means ± SE of three replicates. *** represent $p \leq 0.05$.

4. Discussion

Grafting scion cultivars onto rootstocks is utilized to manipulate plant adaptation to a range of stresses, thereby improving growth and development of vegetables [14,15,19,29]. According to a previous study, grafting watermelon onto *Cucurbita maxima* and *Cucurbita moschata* (pumpkin) rootstocks enhanced the dry matter accumulation under both limited and optimum N availability [4,14]. The authors attributed this increased dry matter accumulation to the enhanced supply of nitrogen and other macro- and micronutrients, such as calcium, phosphorous, copper, manganese, and zinc, and cytokinins. However, they did not investigate the effect of wild watermelon, and also did not present data regarding the photosynthetic responses. Grafting mini-watermelon onto rootstocks also enhanced the plant biomass, nitrogen uptake, and marketable yield [30]. In this study, grafting watermelon onto several wild watermelon rootstocks improved the growth and photosynthetic capacity of the plants compared with the self-grafted watermelon plants (Figures 1 and 2). Use of rootstocks likely altered the leaf structure, mesophyll thickness, and the number of cells in the palisade parenchyma, the spongy parenchyma, and the intercellular spaces [4], thereby improving the photosynthetic assimilation. According to another report, under heavy metal stress conditions, pumpkin rootstock improved the chlorophyll content and rate of photosynthesis of watermelon scion [22]. Similarly, the chlorophyll contents and plant growth of brinjal cultivar "Jiza long" was substantially improved by grafting onto an eggplant rootstock "Hiranasu" under suboptimal temperature conditions [31]. The leaf structure affects the photosynthetic capacity of leaves. The leaf mesophyll conductance is affected by the structure and thickness of the palisade parenchyma and spongy parenchyma [4]. The palisade parenchyma affects light penetration and the spongy parenchyma alters light capture by scattering the light [32–35]. Grafting mini-watermelon onto rootstocks improved the total leaf area and the SPAD index [13]. Pumpkin rootstock improves the relative chlorophyll content and rate of photosynthesis of watermelon, by inducing the expression of the chlorophyll synthesis genes and reducing the expression of the chlorophyll degradation genes [4,22].

The vigorous root system of the rootstocks helps enhance water and ion uptake from the soil solution, and transport to aboveground plant parts [19]. For instance, the watermelon plants grafted onto pumpkin (Jingxinzhen No. 4) rootstock showed 2.24- and 2.02-fold improvement in root dry weight and K-uptake efficiency, respectively, compared with self-grafted transplants [36]. Melon cultivars grafted onto three *Cucurbita maxima* rootstocks (Shintoza, Kamel, and RS-84) showed an improved profile of macronutrients in the leaf tissues. Rootstocks enhanced the N concentration (6% to 81%) of leaves compared with transplants [37]. Similarly, watermelon cultivar "Crimson Tide" grafted onto gourd rootstocks (*Lagenaria siceraria*) apparently increased nitrogen and potassium absorption from the soil under salt stress conditions [38]. Grafting of watermelon cultivar Zaojia 8424 onto pumpkin cultivar "Qingyanzhen No. 1" and bottle gourd cultivar "Jingxinzhen No. 1" increased the total ion absorption of watermelon by up to 30% and 49% at fruit development, and 21% and 47% at fruit maturity, compared with self-rooted plants [39]. Pumpkin-grafted watermelon plants also showed maximum magnesium uptake compared with self-rooted watermelon plants under limited magnesium supply [40].

The improvement of NUE efficiency is required, and the use of rootstocks has been reported to enhance the NUE in different fruit and vegetable crops, including watermelon [4,15,17,21,36,40–42]. In this study, we observed that the N uptake efficiency, N utilization efficiency, and N use efficiency of watermelon was altered by the use of wild watermelon rootstocks (Figure 3a–c). The improved N use efficiency may be attributed to the N uptake efficiency of wild watermelon rootstocks because the uptake efficiency of ZXG-516, ZXG-1558, ZXG-945, and ZXG-952 was better compared with other rootstocks, and thus the N use efficiency of these rootstock–scion combinations was also better (Figure 3a,c). Watermelon grafting onto rootstocks enhanced the nitrogen uptake efficiency and nitrogen use efficiency by 21% and 38%, respectively [30]. According to some previous studies, rootstocks enhance nutrient [14,39,40] and cytokinin supply to the scion, improve growth and dry matter accumulation, and trigger the gene expression of nitrate reductase and nitrite reductase enzymes [4,22,43]. In fruit crops, such as grapes, nitrate uptake is favored by the presence of rootstocks and the characteristics of the scion. The overall nitrate acquisition is affected by low affinity nitrate uptake and efflux from the root cells [44]. Grafting mini-watermelon onto rootstocks increased the activity of nitrate reductase under different levels of nitrogen supply [30]. Thus, the activity of nitrite reductase and nitrate reductase is improved [45], thereby, leading towards the enhanced N assimilation capacity of grafted plants. Interestingly, for some rootstocks (ZXG-941, ZXG-1251, ZXG-1469, and ZXG-1463), their N uptake efficiency was lower compared with other rootstocks but their capacity to utilize the available N was better, thus their N utilization efficiency was improved. The improved nitrate reductase activity [4,30] probably helped the grafted watermelon plants to improve N assimilation and nitrogen use efficiency, particularly at low N supply.

5. Conclusions

This is the first study in which the assessment of wild watermelon genotypes to improve NUE of watermelon has been focused. NUE of watermelon can be improved using wild watermelon rootstocks. Wild watermelon rootstocks improved the NUE by improving the nitrogen uptake, relative chlorophyll content, rate of photosynthesis, and biomass and N allocation in the scion. Based on the results of this study, ZXG-945, ZXG-1250, and ZXG-941 can be used as nitrogen efficient rootstocks under optimum N supply, and ZXG-1558 and ZXG-516 under low N supplies. This study can aid in the selection of better wild watermelon rootstocks for efficient N utilization. However, the response of these rootstocks on reproductive behavior and fruit quality parameters requires further investigations. Additionally, genome-wide transcriptome analysis may help understand the underlying genetic regulation mechanisms [46] involved in N uptake and its utilization.

Author Contributions: X.H., M.A.N., C.C., Z.Z., and F.S. conducted research work, collected and analyzed the data, and prepared the manuscript. Z.B. and Y.H. designed and supervised this study, and revised and approved this manuscript for publication.

Funding: This research was funded by The National Key Research and Development Program of China (2018YFD0201200), National Natural Science Foundation of China (31471919), China Agriculture Research System (CARS-25), and The Fundamental Research Funds for the Central Universities (2662018JC037).

Conflicts of Interest: All the authors declare no conflicts of interest.

References

1. Masclaux-Daubresse, C.; Daniel-Vedele, F.; Dechorgnat, J.; Chardon, F.; Gaufichon, L.; Suzuki, A. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Ann. Bot.* **2010**, *105*, 1141–1157. [CrossRef] [PubMed]
2. Prinsi, B.; Negri, A.S.; Pesaresi, P.; Cocucci, M.; Espen, L. Evaluation of protein pattern changes in roots and leaves of *Zea mays* plants in response to nitrate availability by two-dimensional gel electrophoresis analysis. *BMC Plant Biol.* **2009**, *9*, 113. [CrossRef] [PubMed]
3. Curci, P.L.; Cigliano, R.A.; Zuluaga, D.L.; Janni, M.; Sanseverino, W.; Sonnante, G. Transcriptomic response of durum wheat to nitrogen starvation. *Sci. Rep.* **2017**, *7*, 1176. [CrossRef] [PubMed]
4. Nawaz, M.A.; Wang, L.; Jiao, Y.Y.; Chen, C.; Zhao, L.; Mei, M.; Yu, Y.; Bie, Z.; Huang, Y. Pumpkin rootstock improves nitrogen use efficiency of watermelon scion by enhancing nutrient uptake, cytokinin content, and expression of nitrate reductase gene. *Plant Growth Regul.* **2017**, *82*, 233–246. [CrossRef]
5. Luo, J.; Li, H.; Liu, T.; Polle, A.; Peng, C.; Luo, Z.B. Nitrogen metabolism of two contrasting poplar species during acclimation to limiting nitrogen availability. *J. Exp. Bot.* **2013**, *64*, 4207–4224. [CrossRef] [PubMed]
6. Xin, M.; Wang, L.; Liu, Y.; Feng, Z.; Zhou, X.; Qin, Z. Transcriptome profiling of cucumber genome expression in response to long-term low nitrogen stress. *Acta Physiologiae Plantarum.* **2017**, *39*, 130. [CrossRef]
7. Hakeem, K.R.; Ahmad, A.; Iqbal, M.; Gucel, S.; Ozturk, M. Nitrogen-efficient rice cultivars can reduce nitrate pollution. *Environ. Sci. Poll. Res.* **2011**, *18*, 1184–1193. [CrossRef] [PubMed]
8. Sattelmacher, B.; Horst, W.J.; Becker, H.C. Factors that contribute to genetic variation for nutrient efficiency of crop plants. *J. Plant Nutr. Soil Sci.* **1994**, *157*, 215–224. [CrossRef]

9. Gerendás, J.; Abbadi, J.; Sattelmacher, B. Potassium efficiency of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.). *J. Plant Nut Soil Sci.* **2008**, *171*, 431–439. [CrossRef]
10. Meister, R.; Rajani, M.S.; Ruzicka, D.; Schachtman, D.P. Challenges of modifying root traits in crops for agriculture. *Trends Plant Sci.* **2014**, *19*, 779–788. [CrossRef] [PubMed]
11. Rogers, E.D.; Benfey, P.N. Regulation of plant root system architecture: implications for crop advancement. *Curr. Opin. Biotechnol.* **2015**, *32*, 93–98. [CrossRef] [PubMed]
12. Wissuwa, M.; Kretzschmar, T.; Rose, T.J. From promise to application: Root traits for enhanced nutrient capture in rice breeding. *J. Exp. Bot.* **2016**, *67*, 3605–3615. [CrossRef] [PubMed]
13. Colla, G.; Suarez, C.M.C.; Cardarelli, M. Improving Nitrogen Use Efficiency in Melon by Grafting. *HortScience* **2010**, *45*, 559–565.
14. Nawaz, M.A.; Imtiaz, M.; Kong, Q.; Fei, C.; Ahmed, W.; Huang, Y.; Bie, Z. Grafting: A technique to modify ion accumulation in horticultural crops. *Front. Plant Sci.* **2016**, *7*, 1457. [CrossRef] [PubMed]
15. Bie, Z.; Nawaz, M.A.; Huang, Y.; Lee, J.M.; Colla, G. Introduction of vegetable grafting. In *Vegetable Grafting: Principles and Practices*; Colla, G., Alfocea, F.P., Schwarz, D., Eds.; CABI Publishing: Oxfordshire, UK, 2017; pp. 1–21.
16. Shireen, F.; Nawaz, M.A.; Chen, C.; Zhang, Q.; Zheng, Z.; Sohail, H.; Sun, J.; Cao, H.; Huang, Y.; Bie, Z. Boron: Functions and approaches to enhance its availability in plants for sustainable agriculture. *Int. J. Mol. Sci.* **2018**, *19*, 1856. [CrossRef] [PubMed]
17. Pulgar, G.; Villora, G.; Moreno, D.A.; Romero, L. Improving the mineral nutrition in grafted watermelon: Nitrogen metabolism. *Biol. Plant.* **2000**, *43*, 607–609. [CrossRef]
18. Zen, Y.A.; Zhu, Y.L.; Huang, B.J.; Yang, L.F. Effects of *Cucurbita ficifolia* as rootstock on growth, fruit setting, disease resistance and leaf nutrient element contents in *Cucumis sativus*. *J. Plant Resour. Environ.* **2004**, *13*, 15–19.
19. Lee, J.M.; Kubota, C.; Tsao, S.J.; Bie, Z.; Echevarria, P.H.; Morra, L.; Oda, M. Current status of vegetable grafting: diffusion, grafting techniques, automation. *Sci. Hortic.* **2010**, *127*, 93–105. [CrossRef]
20. Schwarz, D.; Öztekin, G.B.; Tüzel, Y.; Brückner, B.; Krumbein, A. Rootstocks can enhance tomato growth and quality characteristics at low potassium supply. *Sci. Hortic.* **2013**, *149*, 70–79. [CrossRef]
21. Nawaz, M.A.; Shireen, F.; Huang, Y.; Zhilong, B.; Ahmed, W.; Saleem, B.A. Perspectives of vegetable grafting in Pakistan, current status, challenges and opportunities. *Int. J. Agric. Biol.* **2017**, *19*, 1165–1174. [CrossRef]
22. Nawaz, M.A.; Chen, C.; Shireen, F.; Zheng, Z.; Jiao, Y.; Sohail, H.; Afzal, M.; Imtiaz, M.; Ali, M.A.; Huang, Y.; et al. Improving vanadium stress tolerance of watermelon by grafting onto bottle gourd and pumpkin rootstock. *Plant Growth Regul.* **2018**, *85*, 41–56. [CrossRef]
23. Gregory, P.J.; Atkinson, C.J.; Bengough, A.G.; Else, M.A.; Fernandez-Fernandez, F.; Harrison, R.J.; Schmidt, S. Contributions of roots and rootstocks to sustainable, intensified crop production. *J. Exp. Bot.* **2013**, *64*, 1209–1222. [CrossRef] [PubMed]
24. Albacete, A.; Martinez-Anujar, C.; Marinez-Pérez, A.; Thompson, A.J.; Dodd, I.C.; Pérez-Alfocea, F. Unravelling rootstock×scion interactions to improve food Security. *J. Exp. Bot.* **2015**, *66*, 2211–2226. [CrossRef] [PubMed]
25. Venema, J.H.; Elzenga, J.T.M.; Bouwmeester, H.J. Selection and breeding of robust rootstocks as a tool to improve nutrient-use efficiency and abiotic stress tolerance in tomato. In Proceedings of the First International Conference on Organic Greenhouse Horticulture, Bleiswijk, The Netherlands, 11–14 October 2010; Dorais, M., Bishop, S.D., Eds.; Acta Horticulturae: Leuven, Belgium, 2011.
26. Hassell, R.L.; Memmott, F.; Liere, D.G. Grafting methods for watermelon production. *Hort. Sci.* **2008**, *43*, 1677–1679.
27. Bremner, J.M. Total nitrogen. In *Methods of Soil Analysis*; Part 2, 9; Black, C.A., Evans, D.D., Eds.; American Society of Agronomy: Madison, WI, USA, 1965; pp. 1149–1178.
28. Rengel, Z.; Damon, P.M. Crops and genotypes differ in efficiency of potassium uptake and use. *Physiol. Plant.* **2008**, *133*, 624–636. [CrossRef] [PubMed]
29. Davis, A.R.; Perkins-Veazie, P.; Sakata, Y.; López-Galarza, S.; Maroto, J.V.; Lee, S.G.; Huh, Y.C.; Sun, Z.; Miguel, A.; King, S.R; et al. Cucurbit grafting. *Critic. Rev. Plant Sci.* **2008**, *27*, 50–74. [CrossRef]
30. Colla, G.; Rouphael, Y.; Mirabelli, C.; Cardarelli, M. Nitrogen-use efficiency traits of mini-watermelon in response to grafting and nitrogen-fertilization doses. *J. Plant Nutr. Soil Sci.* **2011**, *174*, 933–941. [CrossRef]

31. Gao, Y.; Tian, Y.; Liang, X.; Gao, L. Effects of single-root-grafting, double-root grafting and compost application on microbial properties of rhizosphere soils in Chinese protected cucumber (*Cucumissativus* L.) production systems. *Sci. Hortic.* **2015**, *186*, 190–200. [CrossRef]
32. Goncalves, B.; Correia, C.M.; Silva, A.P.; Bacelar, E.A.; Santos, A. Leaf structure and function of sweet cherry tree (*Prunusavium* L.) cultivars with open and dense canopies. *Sci. Hortic.* **2008**, *116*, 381–387. [CrossRef]
33. Evans, J.R. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia J.* **1989**, *78*, 9–19. [CrossRef] [PubMed]
34. Evans, J.R.; Kaldenhoff, R.; Genty, B.; Terashima, I. Resistances along the CO2 diffusion pathway inside leaves. *J. Exp. Bot.* **2009**, *60*, 2235–2248. [CrossRef] [PubMed]
35. Fan, X.X.; Xu, Z.G.; Liu, X.Y.; Tang, C.M.; Wang, L.W.; Han, X.L. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. *Sci. Hortic.* **2013**, *153*, 50–55. [CrossRef]
36. Huang, Y.; Li, J.; Hua, B.; Liu, Z.; Fan, M.; Bie, Z. Grafting onto different rootstocks as a means to improve watermelon tolerance to low potassium stress. *Sci. Hortic.* **2013**, *149*, 80–85. [CrossRef]
37. Ruiz, J.M.; Belakbir, A.; Lhpez-Cantarero, I.; Romero, L. Leaf-macronutrient content and yield in grafted melon plants. A model to evaluate the influence of rootstock genotype. *Sci. Hortic.* **1997**, *71*, 227–234. [CrossRef]
38. Uygur, V.; Yetisir, H. Effects of rootstocks on some growth parameters, phosphorous and nitrogen uptake by watermelon under salt stress. *J. Plant Nutr.* **2009**, *32*, 629–643. [CrossRef]
39. Huang, Y.; Zhao, L.Q.; Kong, Q.S.; Cheng, F.; Niu, M.L.; Xie, J.J.; Nawaz, M.A.; Bie, Z.L. Comprehensive mineral nutrition analysis of watermelon grafted onto two different rootstocks. *Hort. Plant J.* **2016**, *2*, 105–113. [CrossRef]
40. Huang, Y.; Jiao, Y.; Nawaz, M.A.; Chen, C.; Liu, L.; Lu, Z.; Kong, Q.; Cheng, F.; Bie, Z. Improving magnesium uptake, photosynthesis and antioxidant enzyme activities of watermelon by grafting onto pumpkin rootstock under low magnesium. *Plant Soil.* **2016**, *409*, 229–246. [CrossRef]
41. Ahmed, W.; Pervez, M.A.; Amjad, M.; Khalid, M.; Ayyub, C.M.; Nawaz, M.A. Effect of stionic combination on the growth and yield of Kinnow mandarin (*Citrus reticulate* Blanco.). *Pak. J. Bot.* **2006**, *38*, 603–612.
42. Ahmed, W.; Nawaz, M.A.; Iqbal, M.A.; Khan, M.M. Effect of different rootstocks on plant nutrient Status and yield in Kinnow mandarin (*Citrus reticulate* Blanco). *Pak. J. Bot.* **2007**, *39*, 1779–1786.
43. Nawaz, M.A.; Jiao, Y.; Chen, C.; Shireen, F.; Zheng, Z.; Imtiaz, M.; Bie, Z.; Huang, Y. Melatonin pretreatment improves vanadium stress tolerance of watermelon seedlings by reducing vanadium concentration in the leaves and regulating melatonin biosynthesis and antioxidant-related gene expression. *J. Plant Physiol.* **2018**, *220*, 115–127. [CrossRef] [PubMed]
44. Tomasi, N.; Monte, R.; Varanini, Z.; Cesco, S.; Pinton, R. Induction of nitrate uptake in Sauvignon Blanc and Chardonnay grapevines depends on the scion and is affected by the rootstock. *Aust. J. Grape Wine Res.* **2015**, *21*, 331–338. [CrossRef]
45. Yang, Y.; Lu, X.; Yan, B.; Li, B.; Sun, J.; Guo, S.; Tezuka, T. Bottle gourd rootstock-grafting affects nitrogen metabolism in NaCl-stressed watermelon leaves and enhances short-term salt tolerance. *J. Plant Physiol.* **2013**, *170*, 653–661. [CrossRef] [PubMed]
46. Nawaz, M.A; Chen, C.; Shireen, F.; Zheng, Z.; Sohail, H.; Afzal, M.; Ali, M.A.; Zhilong, B.; Huang, Y. Genome-wide expression profiling of leaves and root of watermelon in response to low nitrogen. *BMC Genomics* **2018**, *19*, 456. [CrossRef] [PubMed]