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Rht13 DWARFING GENE DELAYS FOLIAR SENESCENCE IN WHEAT INDUCED BY NITROGEN DEFICIENCY

NINGNING ZHANG[1], JIAKUN YAN[1*] AND SUIQI ZHANG[2]

[1]Shaanxi Key Laboratory of Ecological Restoration in Shanbei Mining Area, College of Life Science, Yulin University, Yulin, Shaanxi, China
[2]Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi, China
[*]Corresponding author's emails: himingse@163.com

Abstract

Nitrogen is an essential mineral nutrient for maintaining plant growth, development and reproduction. Nitrogen deficiency is one of the most serious abiotic stresses affecting grain yiled production around the world. We studied the effect of the Rht13 dwarfing gene on the physiological response of hydroponically grown wheat to nitrogen deficiency. The Rht13 wheat was able to maintain a higher photosynthetic rate than the wild-type rht genotype under conditions of low nitrogen. Foliar senescence was slower for the Rht13 than the rht wheat, so the Rht13 leaves contained more nitrogen and chlorophyll and had a higher actual photochemical efficiency and photosynthetic rate. These advantages directly led to more accumulation of whole-plant biomass in the Rht13 than the rht wheat under conditions of low nitrogen. The Rht13 gene can thus be potentially used for breeding wheat for cultivation in infertile soil.

Key words: Nitrogen, Wheat, Rht13, Physiological response.

Introduction

Crop growth and development require more nitrogen (N) than other mineral nutrients. Access to, and application of, N fertilizers are very unequally distributed among regions of the world (Davidson, 2009; Tilman *et al*., 2011; Chen *et al*., 2014). N deficiency is a major abiotic stress that reduces yield in the temperate zone, especially in arid and semiarid regions. Environmental pollution from nitrates leaching into the groundwater and the emission of greenhouse gas like nitrous oxide into the atmosphere, however, can supply large amounts of N. Low-input strategies for N fertilization thus need to be developed. Breeding N-efficient cultivars is an important and efficacious strategy under N-deficit conditions (Presterl *et al*., 2003).

Wheat is the leading source of vegetable protein in human food globally. Wheat production has increased substantially in past decades, especially after the introduction of dwarfing (reduced height, Rht) genes (Evenson & Gollin, 2003; Hedden, 2003; Guarda *et al*., 2004; Sun *et al*., 2014). Rht genes can affect wheat grain quality and resistance to abiotic stress (Alghabari *et al*., 2014; Kocheva *et al*., 2014; Casebow *et al*., 2016). The introduction of dwarfing genes has been demonstrated to increase drought resistance and water-use efficiency in wheat cultivars (Yan & Zhang, 2017). Dwarfing genes can also affect phosphorus (P) uptake and grain P content (Manske *et al*., 2002). Modern wheat varieties released after 1970 have intrinsic traits capable of ensuring grain yield and quality and have improved N uptake and use when the N supply is low (Guarda *et al*., 2004). Most modern wheat cultivars contain dwarfing genes, so the improved N uptake and use efficiency may be affected by Rht genes.

The Rht13 dwarfing gene in wheat, located on the short arm of chromosome 7B and derived from the Rht13 bread-wheat donor Magnif M1, reduces peduncle length and plant height to increase grain number and grain yield (Ellis *et al*., 2005; Rebetzke *et al*., 2011). Rht13 has a greater effect on plant height than both Rht-B1b and Rht-D1b (Rebetzke *et al*., 2012). Recent studies of Rht13 have focused on plant height and agronomic traits under natural conditions (Rebetzke *et al*., 2011; Wang *et al*., 2014; Wang *et al*., 2015). The Rht-B1b gene improves N-use efficiency in wheat (Loddo & Gooding, 2012), but little is known about the effects of Rht13 on wheat N-use efficiency.

We hypothesized that the Rht13 dwarfing gene would improve the capacity to maintain growth under conditions of N deficiency. We used a set of recombinant inbred lines to determine 1) the accumulation of aboveground and root biomass, 2) photosynthetic rate and chlorophyll fluorescence and 3) the N contents of leaves and roots. Clarification of these aspects may help to simplify and/or improve experimental procedures for evaluating N-use efficiency in short-term experiments.

Materials and Methods

Plant materials and growing conditions: We used recombinant inbred lines developed from the hybridization of the bread-wheat variety 'Jinmai47' and the Rht13 donor Magnif M1. The materials were developed by the College of Agronomy, Northwest A&F University in China (Wang *et al*., 2015). The varieties were crossed in May 2009, and the F1 plants were self-pollinated to generate an F2 population (296 in total). The presence or absence of the Rht13 dwarfing gene was determined using molecular markers. Similar growth periods, grain yields and plant heights were used to select three Rht13 lines and two wild-type rht lines.

Sterilized seeds were vernalized at 4°C for 24 h and then germinated in the dark at 25°C. Seedlings of similar size were transplanted into pots, which was well aerated using aquarium diffusers, and were grown hydroponically in a growth chamber under a 12/12 h photoperiod (25/18°C, RH=50–60%, 400 μmol photons m^{-2} s^{-1}) in modified Hoagland's nutrient solution (pH 6.0) (Yan *et al*., 2016). The nutrient solution contained 2.4 mM $Ca(NO_3)_2 4H_2O$, 1.6 mM KNO_3, 0.5 mM $(NH_4)_2SO_4$, 1 mM KH_2PO_4, 1 mM $MgSO_4 7H_2O$, 5 μM Fe–EDTA, 2.35 μM H_3BO_3, 0.55 μM $MnSO_4H_2O$, 0.0385 μM $ZnSO_4 7H_2O$, 0.0165 μM $CuSO_4 5H_2O$ and 0.0065 μM H_2MoO_4. The experiment tested two N (1:4 NH^{4+}:NO^{3-}) concentrations: 5 mM N (control, CK) and 0.25 mM N (N deficiency, ND). The levels of calcium and potassium

were balanced between the two treatments using K_2SO_4, $CaCl_2$ and $CaSO_4$. The experiment had a completely randomized block design with five replicates.

Measurements and plant analysis: Measurements were recorded eight days (fully unfolded second leaf) and 25 days (senescent second leaf) after transplantation. Plants were separated into shoots and roots. The samples were dried at 70°C to a constant weight and then weighed. Biomass was measured only when the second leaf began to senesce.

Five replicate foliar discs were ground in liquid nitrogen and extracted in 80% acetone. This solution was centrifuged with 7000 g for 10 min at room temperature, and the absorbance of the supernatant was measured spectrophotometrically at wavelengths of 664 and 647 nm. Chlorophyll a and b (Chl a and Chl b) contents were calculated based on foliar area as described by Lichtenthaler (1987).

Photosynthetic rate was measured between 10:00 and 11:00 under ambient conditions using an Li-6400 portable photosynthesis system (Li-COR Biosciences, Lincoln, USA). The second leaf was placed in the chamber under a photon flux density of 500 µmol m^{-2} s^{-1}. The flow rate through the chamber was 500 µmol s^{-1}, and the foliar temperature was 25°C. Five replicates of each wheat line in both N treatments were tested.

Chlorophyll fluorescence of the senescent second leaf was measured with a pulse-amplitude-modulated chlorophyll-fluorescence system (Imaging PAM, Walz, Effeltrich, Germany) at room temperature. The maximum (Fv/Fm) and effective quantum yields for photosystem II(PSII) were obtained using Imaging Win (Version 2.40, Walz). Each treatment included four replicates.

Foliar N content was determined using the Kjeldahl procedure with a Kjeltec 2300 analyzer (Foss Tecator AB, Hoganas, Sweden).

An analysis of variance tested for differences between the samples, and treatment means were compared using a least significant difference test, using SPSS 19.0 (SPSS Inc., Chicago, USA). The figures were drafted with SigmaPlot 12.5 (Systat Software Inc., San Jose, USA). Means and standard deviations are reported.

Results

Biomass and photosynthetic rate: Root dry weight was significantly higher for the Rht13 than the rht lines in the control treatment (p<0.05, Fig. 1), and similar results were also obtained in the N-deficit treatment. Shoot dry weight was significantly lower in the N-deficit than the control treatment (p<0.05) and was higher for the Rht13 than the rht lines, but significantly only in the control treatment. Whole-plant dry weight was higher for the Rht13 than the rht lines in both treatments. The root/shoot ratio was higher in the N-deficit than the control treatment but did not differ between the two genotypes (Fig. 1).

Photosynthetic rate was significantly higher in the Rht13 than the rht lines (p<0.05) in both treatments and in both fully unfolded and senescent second leaves (Fig. 2).

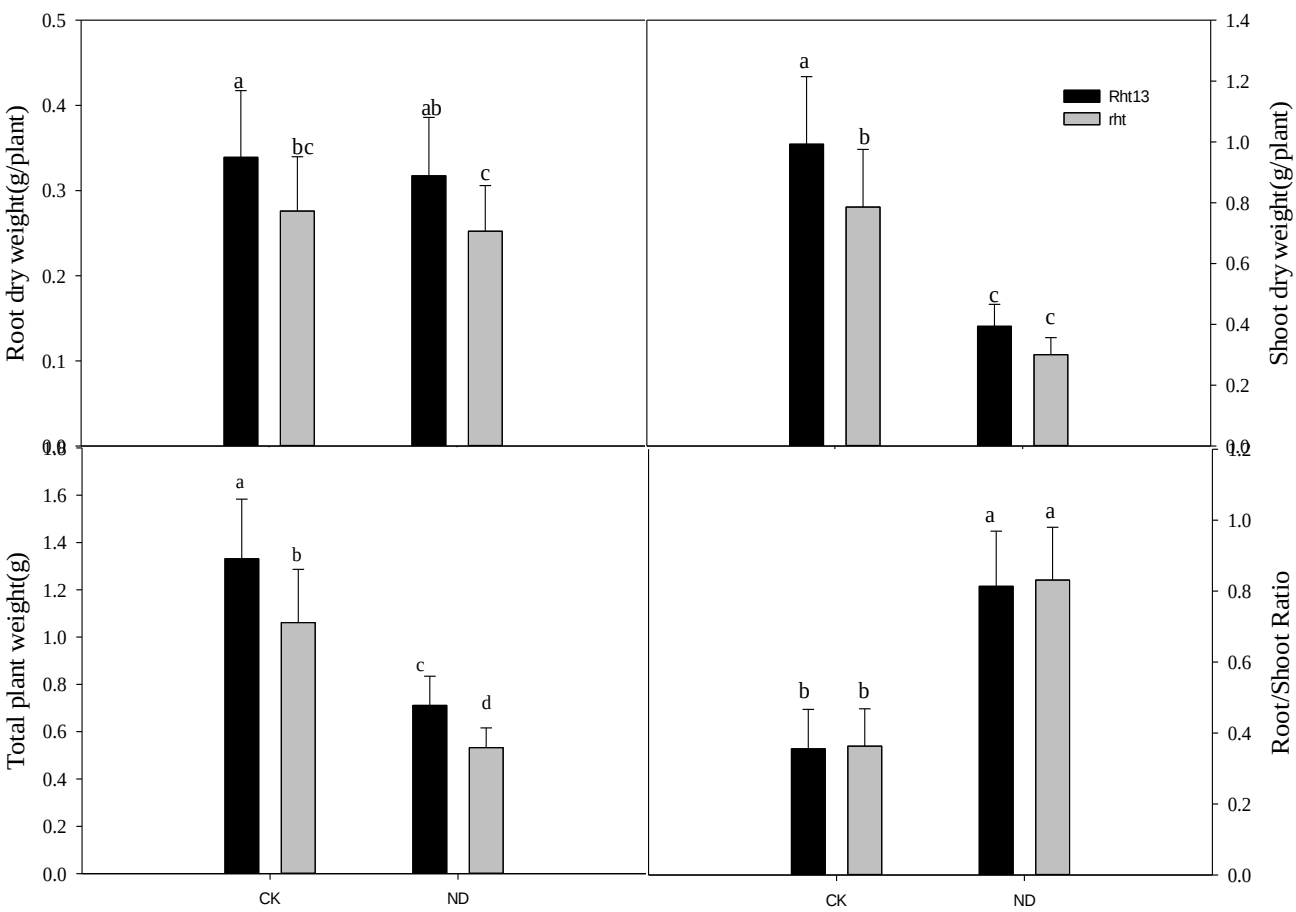

Fig. 1. Effect of N deficiency on the two wheat genotypes.

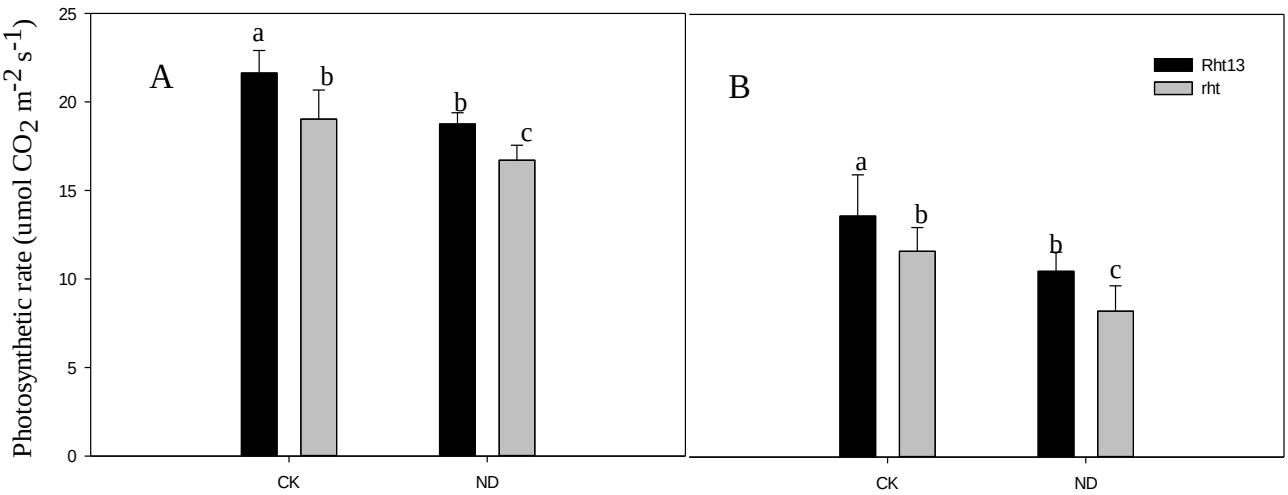

Fig. 2. Effect of N deficiency on photosynthetic rate of the two wheat genotypes (A) eight and (B) 25 days after transplantation.

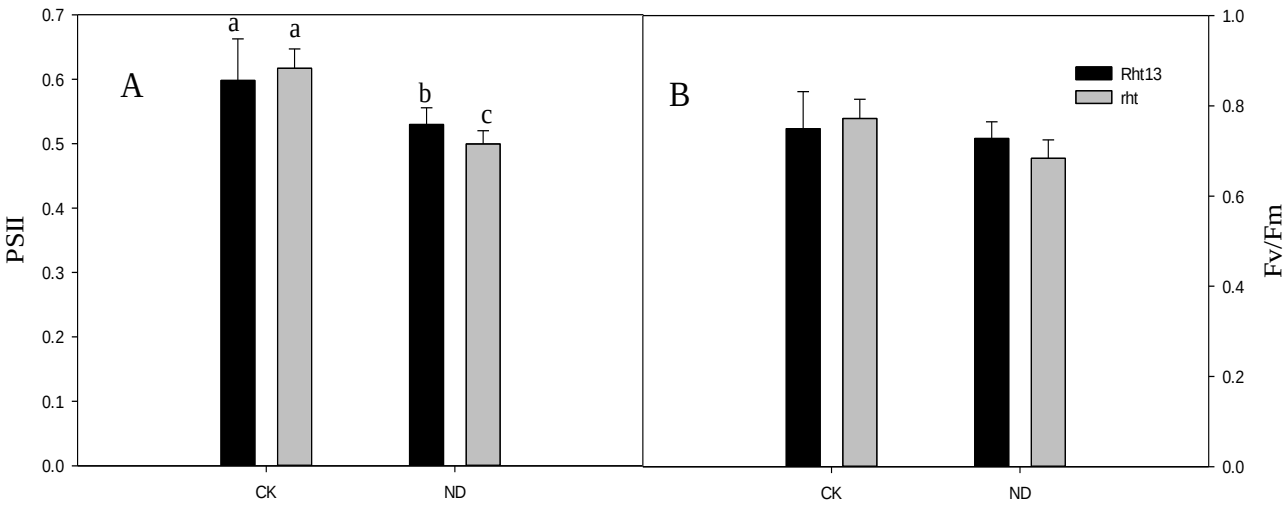

Fig. 3. Effect of N deficiency on fluorescence of the two wheat genotypes after 25 days transplantation.

Fluorescence and foliar chlorophyll content: Fv/Fm in the N-deficit treatment was higher in the Rht13 than the rht lines, but not significantly (p>0.05, Fig. 3). PSII was higher in the Rht13 than the rht lines in the N-deficit but not the control treatment.

Foliar chlorophyll content in the N-deficit treatment changed with foliar senescence (Table 1). Chl a content was 51.7 and 65.1% lower in the Rht13 and rht lines, respectively, in the senescent than the fully unfolded second leaves. Chl b content was 64.6 and 78% lower in the Rht13 and the rht lines, respectively, in the senescent than the fully unfolded second leaves. Total foliar chlorophyll content was higher in the Rht13 than the rht lines in the N-deficit treatment, suggesting that the Rht13 gene delayed the senescence of old leaves.

Foliar N content was lower in the N-deficit than the control treatment, by 45.0% in the Rht13 lines and by 46.1% in the rht lines, but did not differ significantly between the genotypes in either treatment (Fig. 4). Foliar N content was lower in the Rht13 than the rht lines in the fully unfolded second leaves but higher in the senescent second leaves.

Discussion

This study determined the potential of the Rht13 dwarfing gene for maintaining the growth of wheat seedlings to improve our understanding of the effect of Rht13 on wheat adaptation and selecting for adaptation to specific N-deficit conditions.

Root/shoot ratio and N uptake: Roots take up soil water and nutrients, and abiotic stresses such as water and nutrient deficits can increase the root/shoot ratio (Bonifas *et al*., 2005; Yin *et al*., 2014; Sainju *et al*., 2017). The N-deficit treatment in our study dramatically increased the root/shoot ratio, likely due to the allocation of more biomass to the roots than the shoots. Allocation responses are strongest when nutrients are limiting, with a large increase in root biomass at the expense of stem and especially foliar biomass (Poorter *et al.*, 2012). Sunflower seedlings allocated more biomass to roots under salinity stress (Ma *et al.*, 2017). The allocation of more biomass to the roots of our wheat seedlings, producing a higher root/shoot ratio, would thus increase the ability to take up N under N-deficit conditions. The root/shoot ratio was not higher in the Rht13 than the rht seedlings, so the Rht13 seedlings did not have an increased ability to take up N.

Table 1. Effect of N deficiency on chlorophyll content of two wheat genotypes eight and 25 days after transplantation.

		Eight days			Twenty-five days		
		Chl a	**Chl b**	**Chl a+b**	**Chl a**	**Chl b**	**Chl a+b**
CK	Rht13	1.48 ± 0.13	0.47 ± 0.07	1.94 ± 0.17	1.59 ± 0.47	0.34 ± 0.14	1.93 ± 0.57
	rht	1.46 ± 0.22	0.53 ± 0.08	2.00 ± 0.29	1.47 ± 0.13	0.32 ± 0.02	1.80 ± 0.15
ND	Rht13	1.43 ± 0.20	0.48 ± 0.09	1.91 ± 0.27	0.69 ± 0.08	0.17 ± 0.03	0.86 ± 0.13
	rht	1.46 ± 0.14	0.50 ± 0.05	1.96 ± 0.17	0.51 ± 0.09	0.11 ± 0.05	0.62 ± 0.13

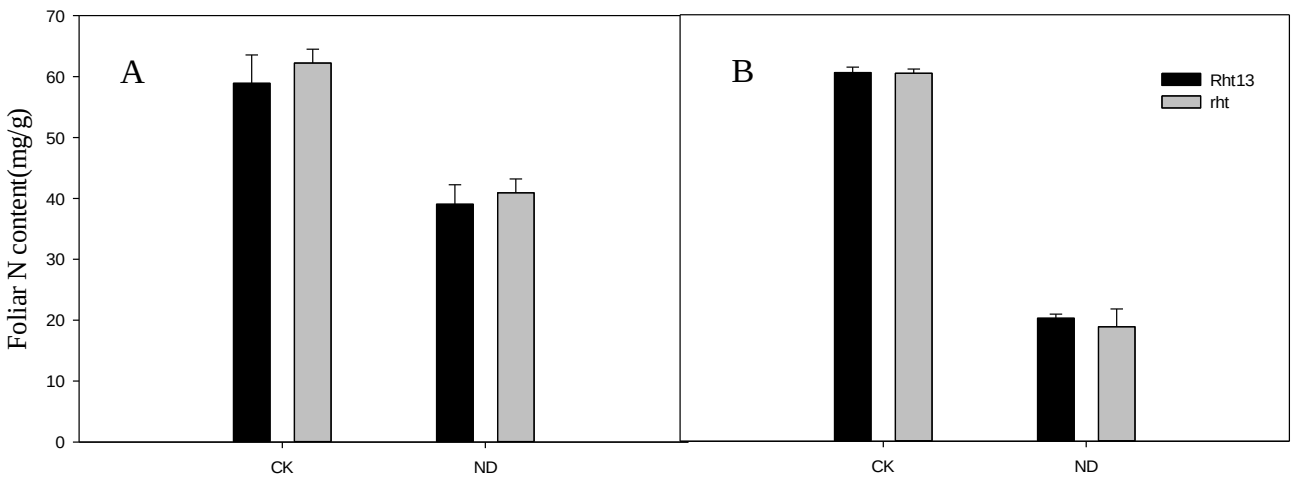

Fig. 4. Effect of N deficiency on foliar N content of the two wheat genotypes (A) eight and (B) 25 days after transplantation.

Photosynthetic rate and fluorescence: N plays an important role in maintaining foliar photosynthetic rate (Liu *et al.*, 2016). Lower photosynthetic electron transport capacity, foliar chlorophyll content and initial rubisco activity under N deficiency can decrease the photosynthetic rate (Lin *et al*., 2016). The photosynthetic rate in the N-deficit treatment in our study was higher in the Rht13 than the rht lines in both the fully unfolded second and senescent leaves (Fig. 2). The Rht13 leaves also had a better capacity to maintain higher Fv/Fm and PSII. The Rht13 lines were thus better adapted to N deficiency than the rht lines. Lower photosynthetic rate, chlorophyll content and Fv/Fm, however, are symptoms of foliar senescence (Chen *et al*., 2015).

N remobilization and foliar senescence: Abiotic stress modulates plant senescence, and foliar senescence plays an important role in maintaining nutrient recycling and thus modulates crop plant growth, development and reproduction significantly (Pandey *et al.*, 2017). N remobilization induced by the senescence of old leaves can translocate N into new leaves under N-deficit conditions, which is very important for plant growth (Han *et al.*, 2017). The capacity of a genotype in maintaining a higher photosynthetic capacity of older leaves during senescence induced by N deficiency at the seedling stage is a suitable selection parameter for N-use efficiency of tropical maize cultivars, indicating that delayed senescence induced by N deficiency in old leaves could be an important indicator for selecting for N-use efficiency in crop cultivars (Schulte auf'm Erley *et al*., 2007). The remobilization of N in the older leaves in our study was slower in the Rht13 than the rht lines (Fig. 4). This slow N remobilization could allow leaves to retain higher chlorophyll contents and to slow chloroplast senescence (Lim *et al*., 2007). The Rht13 wheat lines alleviated the foliar senescence induced by N deficiency, because foliar N content, Fv/Fm and PSII and chlorophyll content were higher in the older, senescent second leaves, compared to the rht wheat lines (Figs. 3 and 4, and Table 1).

Conclusions

Foliar senescence in the N-deficit treatment was slower for the Rht13 than the rht wheat genotype, so the leaves had higher N and chlorophyll contents and higher actual photochemical efficiencies and photosynthetic rates. These advantages directly led to more accumulation of whole-plant biomass in the Rht13 than the rht wheat genotype under conditions of low N. All these results indicate that the Rht13 gene has the potential to be used for breeding wheat for cultivation in infertile soil.

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