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Short Communication

# Optimized nitrogen fertilizer application mode increased culms lignin accumulation and lodging resistance in culms of winter wheat

Xiaoguang Chen[a,*], Jing Wang[a], Zhenlin Wang[b,*], Wenyang Li[c], Chengyu Wang[d], Suhui Yan[c], Hongmin Li[a], Aijun Zhang[a], Zhonghou Tang[a], Meng Wei[a]

[a] Xuzhou Institute of Agricultural science of the Xuhuai District of Jiangsu Province, Xuzhou 221121, Jiangsu, PR China
[b] State KeyLaboratoryof Crop Biology, Shandong AgricultureUniversity, Tai'an 271018, Shandong, PR China
[c] Agronomy College, Anhui Science and Technology University, Fengyang 233100, Anhui, PR China
[d] Anhui Agriculture University, Hefei 230000, Anhui, PR China

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ABSTRACT

The present study used both winter wheat cultivars Jimai 22 (lodging-resistant cultivar) and Yannong 19 (lodging-susceptible cultivar) to investigate the effect of optimized nitrogen fertilizer application on lignin accumulation, its related enzyme activities in the basal second internode, and the breaking strength and culm lodging resistance index (CLRI) in winter wheat. Results showed that optimized nitrogen fertilizer application rates significantly increased the phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL) and peroxidase (POD) activities as well as lignin accumulation, the breaking strength and CLRI. Lignin accumulation and CLRI could be significantly increased by shifting nitrogen from base to topdressing under identical nitrogen application rates. Lignin accumulation, PAL, TAL and POD activities, the breaking strength and CLRI were significantly increased by an appropriate increase of the booting fertilizer proportion (DC 4.1) when identical base and topdressing ratios. These results suggested that the nitrogen fertilizer application rate of 202.5 kg $hm^{-2}$ with the ratio of 4:4:2 for base (DC 0.0), elongation (DC 3.0) and booting stage (DC 4.1) significantly increased the lodging resistance of winter wheat in Xuhuai district.

## 1. Introduction

Lodging is a common and important problem in the high-yield cultivation of wheat. Lodging causes decreased yield and grain quality, and reduces the efficiency of mechanized harvesting (Pinthus, 1973; Easson et al., 1993; Fischer and Stapper, 1987; Berry et al., 2003; Acreche and Slafer, 2011; Berry and Spink, 2012). Research regarding the lodging resistance of wheat culms has a significant value for the high and stable yield of wheat. Previous studies have indicated the reduction of the plant height as an effective measure to enhance the lodging resistance of culms (Wiersma et al., 1986; Zahour et al., 1987). However, it is difficult to achieve both high yield and lodging resistance by reducing plant height. Therefore, improving the culm quality has become the key factor towards improving lodging resistance (Xiao et al., 2002; Chen et al., 2011). Lignin is a major structural component of cell walls and enhances the mechanical strength of plants; its content is closely related to the obtained lodging resistance (Boudet, 2000; Jones et al., 2001; Ma, 2009, 2010). Lignin-deficient wheat is susceptible to lodging, while increased lignin content enhances the lodging resistance of culms (Welton, 1928; Tripathi et al., 2003; Peng et al., 2014).

Proper nitrogen fertilizer application is essential to ensure high wheat yields. However, irrational nitrogen fertilization impedes the lignification of culms and reduces their quality, while increasing lodging susceptibility (Wei et al., 2008; Chen et al., 2011; Lu et al., 2014). Little or moderate nitrogen application rates increase lignin content in the basal internodes, and enhance lodging resistance, while excessive nitrogen application decreases lignin synthesis related enzyme activities and lignin content, thus reducing lodging resistance (Zadoks et al., 1974; Crook and Ennos, 1995; Entry et al., 1998; Wei et al., 2008; Chen et al., 2011). Therefore, rational nitrogen management strategies may improve the mechanical strength of wheat culms, thus enhancing their lodging resistance. In this study, two wheat cultivars with contrasting lodging resistance were used to evaluate the effects of varying nitrogen application regimes on the synthesis and accumulation of lignin and lodging resistance in wheat culms.

* Corresponding authors.
*E-mail addresses:* chenxggw@163.com (X. Chen), zlwang@sdau.edu.cn (Z. Wang).

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## 2. Material and methods

### 2.1. Plant material and experimental design

During two growing seasons (i.e. 2012–2013 and 2013–2014), Jimai 22 (a lodging-resistant cultivar) and Yannong 19 (a lodging-susceptible cultivar) were used for field trials at the Xuzhou Institute of Agricultural Science in Xuhuai District of Jiangsu Province, China (117°17′E, 34°16′N). The soil at the experimental farm was yellow fluvo-aquic soil, and the test soil layer of 0–20 cm contained 11 g $kg^{-1}$ total organic matter, 72.5 mg $kg^{-1}$ available nitrogen, 58.6 mg $kg^{-1}$ available phosphate and 52.8 mg $kg^{-1}$ available potassium. A split plot design was applied in the experiments. The primary plot was subjected to different nitrogen application rates; local farming fertilization application (270 kg $hm^{-2}$, FP) and optimized fertilization application (202.5 kg $hm^{-2}$, OPT). OPT was based on the nitrogen absorption, the target crop yield and the characteristics of the wheat planting area (Chen et al., 2014). The subplot had three nitrogen allocation modes. A fertilization ratio of 4:4:2 was used for base (DC 0.0), elongation stage (DC 3.0) and booting stage (DC 4.1); a fertilization ratio of 4:6 was used for base (DC 0.0) and elongation stage (DC 3.0), and a fertilization ratio of 6:4 was used for base (DC 0.0) and elongation stage (DC 3.0). The different stages were assigned and represented by a–c, respectively. In total, there were six treatments with three replicates per treatment, and the plot size was 3 × 4 m. Before sowing each plot, 750 kg $hm^{-2}$ of calcium superphosphate and 225 kg $hm^{-2}$ of potassium chloride were applied. Seeds were sown on October 7th 2012 and October 12th 2013, and the basic seedling rate was 2.25 million plants $hm^{-2}$. All other management strategies were consistent with those applied in general high-yield fields.

### 2.2. Sampling

At the beginning of the elongation stage (DC 3.0), c. 300 uniform stems per plot were labelled with red thread. After formation of the basal second internode, 15 of these labelled stems were sampled per plot at 7-day intervals. A total of seven samplings were conducted in total. The basal second internodes of the sampled (with removed stem sheathes) were immediately frozen in liquid nitrogen for at least 30 min and kept at −60 °C for subsequent analysis of lignin content and related enzyme activities. Grain yield and straw yield were both determined from a harvest area of 3 $m^2$ in each plot at maturity.

### 2.3. Lignin determination

Lignin content was quantified according to the method of Syros et al. with modifications (2004). Fresh homogenized stem samples (0.3 g) the were placed in test tubes with 4 ml of 800 ml ethanol/l for 2 h; then the sediments were collected and centrifuged at 4000 × *g* for 10 min. Sediments were extracted via further two successive 2-h extractions in 4 ml of 80% (v/v) ethanol at 80 °C, followed by a 1-h extraction in 4 ml of chloroform at 62 °C. Sediments were then dried for 2 days at 50 °C. Dried sediments were digested in 3.6 ml of 25% (v/v) acetyl bromide solution in acetic acid containing 2.7% (v/v) perchloric acid at 70 °C. After 1 h, 0.3 ml per sample were added to 1.9 ml of a solution consisting of 17.24% (v/v) 2 N sodium hydroxide and 82.76% (v/v) acetic acid, and 0.1 ml of 7.5 mol $L^{-1}$ hydroxylamine hydrochloride were added to terminate the reaction. The volume was corrected with acetic acid to 5 ml total and the absorbance at A280 was measured via spectrophotometer (Shimadzu UV-2450, Tokyo, Japan). Lignin was expressed as $OD_{280}$ $ml^{-1}$ $g^{-1}$ FW.

### 2.4. Enzyme extraction and assays

Stem samples (0.5 g) were homogenized with a pestle in an ice-cold mortar, contained 6 ml buffer (50 mmol $L^{-1}$ sodium phosphate buffer, pH 8.8, 5 mmol $L^{-1}$ 2-mercaptoethanol, 1 mmol $L^{-1}$ Ethylenediaminetetraacetic acid, 0.2 g insoluble polyvinylpolypyrrorolidone [PVPP]) for assay of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) activities (Assis et al., 2001). Stem samples (0.5 g) were homogenized with cold extraction buffer, containing 6 ml of 100 mmol $L^{-1}$ sodium phosphate buffer (pH 7.0) and 0.2 g PVPP for the assay of peroxidase activity (POD) (Moerschbacher et al., 1988). The extracts were filtered through two layers of miracloth and the filtrates were centrifuged at 27,000 × *g* at 4 °C for 30 min.

PAL activity was assayed according to Assis et al. (2001), with slight modifications: 0.2 ml of supernatant was mixed with 2 ml of 50 mmol $L^{-1}$ borate buffer (pH8.8) and 1 ml of 20 mmol $L^{-1}$ 1-phenylalanine and incubated in a water bath at 40 °C for 60 min. The reaction was stopped via heating in boiling water for 1 min. Trans-cinnamate formed in this reaction was quantified with a spectrophotometer (Shimadzu UV-2450, Tokyo, Japan) at 290 nm. One unit of enzyme activity (U) was defined as an increase of 0.01 in absorbance per min. The enzyme activity was expressed as U $mg^{-1}$ protein.

TAL activity was measured in a reaction mixture, in a final volume of 2.5 mL, consisting of 0.2 ml of supernatant, 50 mmol $L^{-1}$ borate buffer (pH8.8) and 5.5 µmol L-tyrosine. The enzyme reaction was started via addition of enzyme extract. After incubation for 60 min at 40 °C, the reaction was stopped via heating in boiling water for 1 min. After cooling, p-coumaric acid that had formed in this reaction was measured at 315 nm. One unit of enzyme activity (U) was again defined as a change of 0.01 in absorbance per min. Enzyme activity was expressed as U $mg^{-1}$ protein (Wajahatullah et al., 2003).

POD activity was assayed according to Moerschbacher et al. (1988). The reaction mixture consisted of supernatant (1 mL) and guaiacol substrate (100 mmol $L^{-1}$ sodium phosphate buffer, pH 7.0 and 20 mmol $L^{-1}$ guaiacol) (3 mL) for POD. The increase in absorbance at 470 nm was measured spectrophotometrically after addition of 20 µL $H_2O_2$. One unit of enzyme activity (U) was defined as a change of 1.0 in absorbance per min. Enzyme activity was expressed as U $mg^{-1}$ protein.

Protein concentrations in the extracts were determined using bovine serum albumin as a standard (Bradford, 1976).

### 2.5. Measurement of breaking strength and culm lodging resistance index (CLRI)

CLRI were measured at the anthesis stage (DC 6.2), the milk stage (DC 7.2) and the dough stage (DC 8.2) following methods reported in Akira et al. (1989) with the follows modifications: the breaking strength of basal second internodes was measured with a plant lodging tester (Hangzhou TOP Instrument Co., Ltd. Hangzhou, China). The stem sheaths of the sampled basal second internodes were removed and the internodes were placed on the groove of the support pillars at a distance of 5 cm. The tester was set perpendicular to the stem at the middle, under gradual loading, and the breaking strength was measured when the culm was pushed to breaking point. The value displayed represents the breaking strength in N. The center of gravity height refers to the distance from the culm (with spike, leaf, and sheath) base to the balance fulcrum. The fresh weight of the shoot was the weight of the ground section of the shoot, with spike, leaf and sheath. Twenty labeled shoots per plot were measured and the mean was calculated at each stage.

The culm lodging resistance index was calculated using the following equations:

Culm lodging resistance index = The breaking strength of basal second internodes/(The culm height at center of gravity × The fresh weight of the shoot).