Journal of Plant Studies; Vol. 2, No. 2; 2013
ISSN 1927-0461 E-ISSN 1927-047X
Published by Canadian Center of Science and Education

# Effect of Nitrogen Application at the Booting Stage on Wheat Progeny Seed Germination and Seedling Growth

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Received: April 24, 2013 Accepted: June 25, 2013 Online Published: August 16, 2013

doi:10.5539/jps.v2n2p158 URL: http://dx.doi.org/10.5539/jps.v2n2p158

**Abstract**

The effects of nitrogen (N) treatment applied at the booting stage on progeny seed germination and seedling growth were investigated in wheat (*Triticum aestivum* L.) cvs. Zheng 9023 (high protein content of 14%) and Yangmai 13 (low protein content of 10%). The N treatment enhanced seed protein content, seedling root length, shoot length, dry weight, chlorophyll contents, activities of superoxide dismutase and dehydrogenase, and cell number and cross-sectional area of roots. N treatment significantly increased progeny seed germination percentage and rate in cv. Yangmai 13, but decreased them in cv. Zheng 9023, relative to controls. N treatment resulted in cell walls and starch granules in seed endosperm degrading faster in cv. Yangmai 13, but more slowly in cv. Zheng 9023, relative to controls. The results will help to provide a practice for improving progeny seedling quality by choosing appropriate varieties and N management.

**Keywords:** wheat seed, nitrogen, physiological indices, structure

**Abbreviations: SOD**, superoxide dismutase; **TTC**, 2,3,5-triphenyl tetrazolium chloride; **DPG**, days post germination; **LM**, light microscopy; **SG,** starch granule; **ER**, embryo; **ED**, endosperm.

## 1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major staple food crops, the second most important cereal crop after maize. Thus, wheat makes a significant contribution to global food production and food safety especially for developing countries (Xia et al., 2012). The status of wheat seed germination and seeding growth significantly affect wheat yields and quality (Zhang et al., 2006; Hung et al., 2011). During wheat seed germination many metabolic enzymes such as cysteine endopeptidases, DNA polymerases and superoxide dismutase (SOD) play important roles in subsequent seedling growth (Ai et al., 2006; Zhang et al., 2007; Chao et al., 2009). Nitrogen (N) is the most important macroelement for wheat development and affects amino acid composition of protein, and hence is often the most limiting factor in crop morphological development, organ formation and yield production (Habash et al., 2007; Cui et al., 2010; Makino, 2011). Many researchers have reported that application of N fertilizer improves not only wheat yields but also protein contents (Su et al., 2003; Souza et al., 2004; Charmet et al., 2005). Application of N fertilizer at different developmental stages has different effects on wheat – generally, application at the booting stage can advance wheat spike differentiation, flowering and fructification, and finally improve wheat quality (Fischer et al., 1993; Hussain et al., 2006; Zhou et al., 2009).

Many studies have been done on the effects of hormones, heavy metals, microelements and other external factors on wheat germination and seedling growth. These studies showed that at lower concentrations these factors had a positive effect on seedling growth, but negative at higher concentrations (Walker-Simmons et al., 1994; Ying et al., 2010; Mostafa et al., 2011; Feizi et al., 2012;). Mo et al. found that N applications at rice booting and tillering stages could increase chlorophyll contents, photosynthetic rate and enzyme activities (Mo et al., 2004). Te et al. reported high protein wheat grain produced heavier seedlings, more total RNA, DNA, amino acids, ribosomes and soluble protein content than that of low protein grain (Ching et al., 1978). Studies have shown that absorption of N by maturing wheat grains can affect the distribution of heavy metals and microelements in grain (Fangmeier et al., 1997; Lintschinger et al., 1997; Perilli et al., 2010) Although there have been many studies on wheat seeds, effects of N application at the booting stage on seed germination and seedling growth have been poorly investigated. In the present study, the physiological and structural effects of N at the booting stage on progeny seed germination and seedling growth were investigated in two wheat varieties with different

protein content. The roles of N in improving progeny seed protein contents and the quality of seedlings were also examined.

## 2. Materials and Methods

### *2.1 Plant Materials*

Wheat cvs. Zhen 9023 (high protein of 14%) and Yangmai 13 (low protein of 10%) were purchased from the National Wheat Improvement Center in Yangzhou, and grown in Yangzhou University experimental field, Yangzhou, Jiangsu, China. The field soil is sandy loam [*Typic fluvaquents,* Entisols (US taxonomy)] which contains organic materials of 2.45% and available nitrogen (N), phosphorus (P) and potassium (K) of 106, 33.8 and 66.4 mg $kg^{-1}$, respectively.

### *2.2 Field Experiment and N Treatment*

The field experiment was conducted on the farm in Yangzhou University in 2011–2012. The field soil is sandy loam containing 14.30 g/kg total N, 45.52 mg/kg available phosphorus, 99.30 mg/kg available potassium and 12.58 g/kg organic materials. At the booting stage each wheat variety received a total of 120 kg N/ha as urea, the control wheat received no N, and all other agronomic practices including land preparation, planting density and field management were kept uniform for all treatments.

### *2.3 Water Culture Experiments*

Only uniformly sized large seeds were used for this study. The plump seeds were surface-sterilized with 1% NaClO for 30 min, then rinsed several times with distilled water and put in water in Petri dishes at 25 °C for 24 h. The seeds were placed on filter paper in culture plates and germinated at 25 °C in an incubator under a light irradiance of 400 mol/$m^2$/s (12/12 h light/dark cycles).The light resource used is electric incandescent lamp. there were three replications per treatment.

### *2.4 Seed Germination and Seedling Growth Experiment*

Seed germination was defined as a root length of ≥ 1.0 mm. Germination rate was determined at 3 days post germination (DPG), and germination percentage determined at 7 DPG. Shoot and root length of seedling axes were measured at 7 DPG, then dry weight was determined after oven-drying at 100 °C. All assays were replicated at least three times to minimize experimental error.

### *2.5 Determination of Chlorophyll Relative Contents*

The chlorophyll relative contents of the first leaf on the seedling axes were determined with a chlorophyll meter (SPAD-502, Hangzhou Top Instrument Co., Ltd) with at least three replications

### *2.6 Determination of SOD Activities of Roots*

SOD activities of roots at 2, 3, 5, 7 and 9 DPG were determined according to Zhang and Li (2007), with at least three replications.

### *2.7 Light Microscopy of Elongation Zone of Root and Grain Structure*

The elongation zone of wheat roots and the middle part of endosperm at 3 DPG were transversely cut with a razor blade and one or two 1.0- to 1.5-mm-thick slices were selected. Each slice was further cut into a number of tissue blocks of 1-2 $mm^2$; tissue blocks were immediately fixed by 2.5% glutaraldehyde, 1% paraformaldehyde and 0.05 mol/L sodium methyl mercaptide. Then, they were fixed by 1% osmic acid. The blocks were washed, dehydrated through an ethanol series of 30-100% and embedded in Spurr's low-viscosity embedding medium. The sections of 1-μm thickness were cut with a glass knife on a Leica Ultracut R (Germany, wetzla), and stained with toluidine blue O for 3 min. The sections were visualized and photographed with a Leica Dmls light microscope (Leica Dmls light microscope Germany, wetzla).

### *2.8 Histochemical Identification in Wheat Pericarp*

Wheat seeds at 3 and 6 DPG were longitudinally cut with a razor blade. Slices of wheat seeds were put on a block of white porcelain. Then, 1% 2,3,5-triphenyl tetrazolium chloride (TTC) solution and I2-KI were added for staining at room temperature, and were photographed using a light microscope (Leica Dmls).

### *2.9 Statistical Analysis*

Each experiment was repeated at least three times. The data were expressed as means ± standard error (SE). Statistical comparisons were carried out using SPSS 13.0 and Excel 2007 software. The cell number and cross-sectional area of roots were calculated with Image J 1.43 software. The significant levels of difference were calculated for all measured traits, and the means compared at $P < 0.05$ and $P < 0.01$.

## 3. Results

### *3.1 Effect of N Treatment on Progeny Grain Protein and Weight*

Most protein in wheat grain is located in the endosperm, and N application at the booting stage increased protein contents of wheat cv. Zheng 9023 by 6.3% and cv. Yangmai 13 by 13.3% over grains produced without N application, and the corresponding grain weight also improved. Moreover, there were highly significant differences between N treatment and controls (Table 1).

Table 1. Protein contents and grain weight of progeny wheat after N application at the booting stage

| Varieties | Treatment | Protein contents (%) | 1000-grain weight(g) |
|---|---|---|---|
| Zheng 9023 | CK | 13.90 A | 53.0 A |
| | N | 14.78 B | 55.4 B |
| Zheng 9023 | CK | 10.24 A | 42.6 A |
| | N | 11.60 B | 44.3 B |

Values within each column marked with different upper case letters indicate a highly significant difference ($p < 0.01$).

### *3.2 Effect of N Treatment on Progeny Seed Germination*

In comparison to controls, N treatment significantly inhibited seed germination in cv. Zheng 9203, and germination percentage and rate decreased by 8.1 and 10.2%, respectively (Table 2). However, in cv. Yangmai 13 the N application promoted germination percentage and rate by 3.2 and 6.5%, respectively.

Compared to controls, N application enhanced root length, shoot length and seedling dry weights (Table 2). N application significantly promoted root length by 14.3 and 13.5% in cvs. Zheng 9023 and Yangmai 13, respectively. N application significantly increased shoot length by 13.7% in cv. Zheng 9023, but the differences were not significant for cv. Yangmai 13. There was no significant effect of N treatment on seedling dry weight.

Table 2. Effect of N application on progeny seed germination of wheat

| Varieties | treatment | Germination percentage (%) | Germination rate(seed day$^{-1}$) | Root length(cm) | Shoot length(cm) | Seedling dry weight(g) |
|---|---|---|---|---|---|---|
| Zheng 9023 | CK | 90.44A | 69.29a | 26.47A | 8.17 A | 0.039a |
| | N | 81.66B | 61.21b | 30.87B | 9.29 B | 0.044a |
| Yangmai 13 | CK | 93.67a | 85.25a | 25.84a | 8.88a | 0.033a |
| | N | 96.67a | 90.78b | 29.88b | 9.12a | 0.036a |

Values within each column marked with different upper case letters indicate a highly significant difference ($p < 0.01$); different lower case letters indicate a significant difference ($p < 0.05$).

### *3.3 Effect of N Application on Seedling Chlorophyll Contents*

In comparison to controls, N application clearly increased the progeny seedling chlorophyll contents – by 12.4%, 4.3% for cv. Zheng 9023 and cv. Yangmai 13, respectively (Figure 1) - this could enhance the photosynthesis rate of leaves.

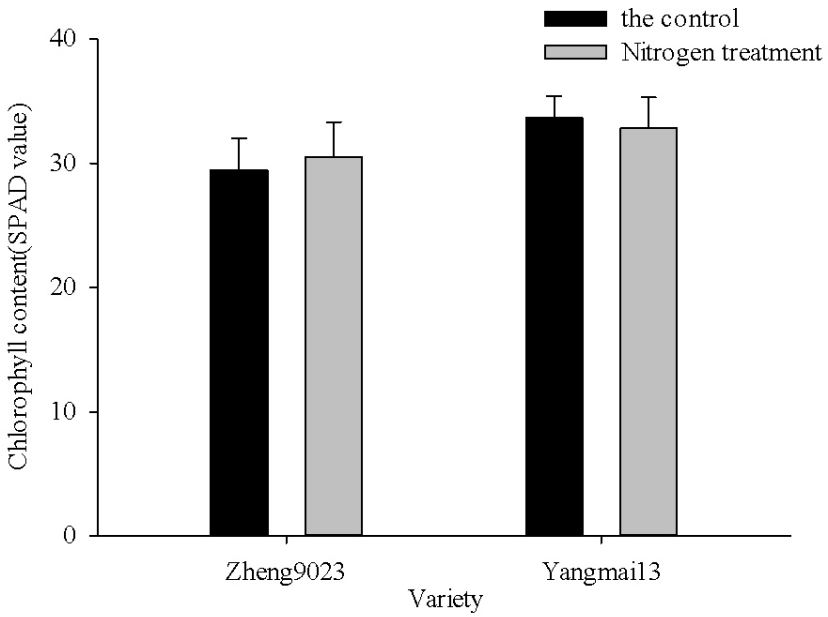


Figure 1. Effect of N treatment on progeny seedling chlorophyll contents

*3.4 Effect of N Treatment on Embryo Dehydrogenase Activity*

Compared to controls, the embryos of the N treatment were stained more deeply by TTC at 3 and 6 DPG, especially in cv. Zheng 9023 (Figure 2), indicating higher dehydrogenase activity in embryos of the N treatment. This also showed the N treatment could prolong and promote dehydrogenase activity, especially at later seed germination stages (Figure 2C, D, G and H).

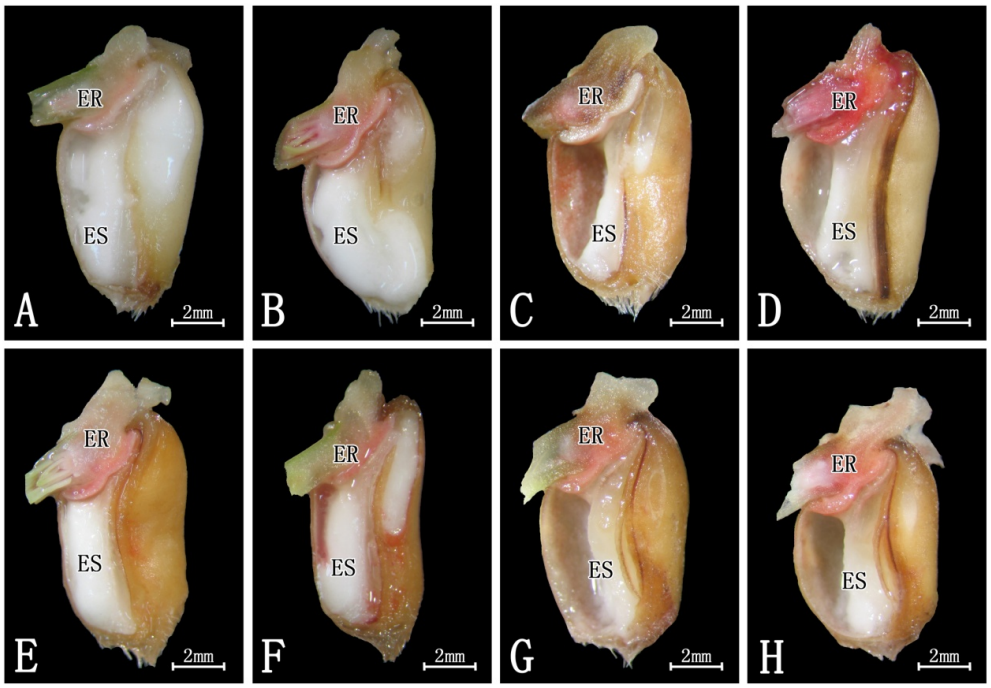


Figure 2. Vertical sections of germinating seeds under LM (×10). A, C: seeds of cv. Zheng 9023 in control at 3 and 6 DPG, respectively; B, D: seeds of cv. Zheng 9023 with N treatment at 3 and 6 DPG, respectively; E, G: seeds of cv. Yangmai 13 in control at 3 and 6 DPG, respectively; F, H: seeds of cv. Yangmai 13 with N treatment at 3 and 6 DPG, respectively. The red color indicates embryo stained by TTC. The white bars indicate 2 mm. ER: embryo; ES: endosperm

*3.5 Effect of N Treatment on Seedling Root SOD Activity*

SOD was investigated during the germination of wheat seeds to discern if activity of enzyme was affected by N treatment. SOD activity of seedling roots was at a maximum at 2 DPG and then gradually decreased (Figures 3 and 4). N application clearly and significantly increased SOD activity of seedling roots, compared to controls, especially up to 3 DPG.

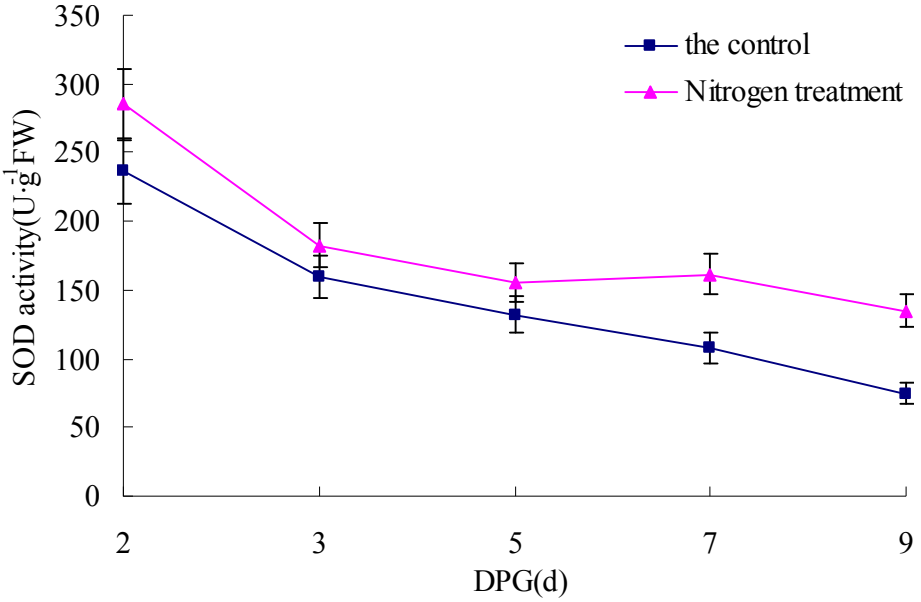


Figure 3. Effect of N treatment on SOD activity of progeny seedling roots of cv. Zheng 9023

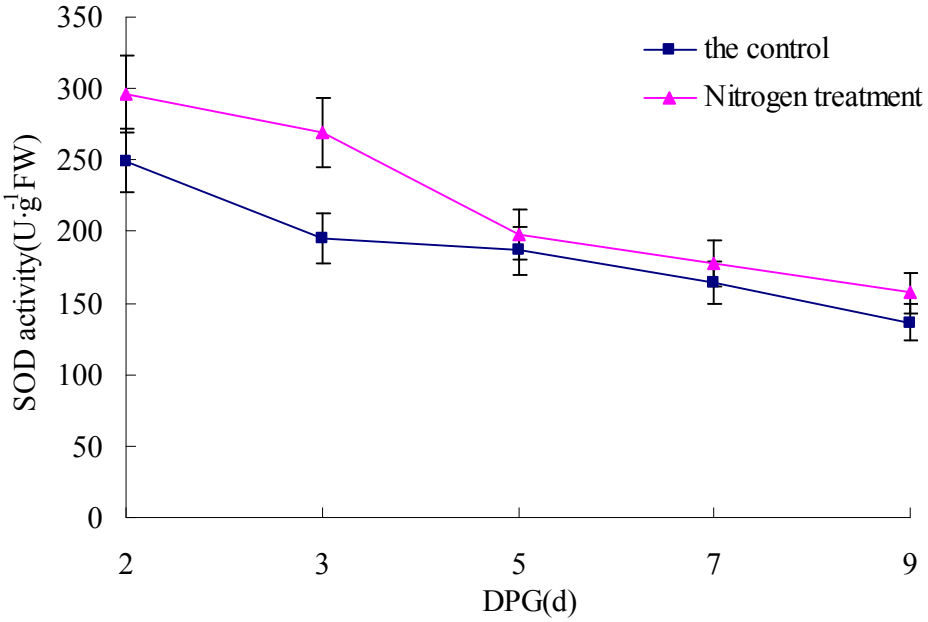


Figure 4. Effect of N treatment on SOD activity of progeny seedling root of cv. Yangmai 13

*3.6 Effect of N Application on Root Elongation Zone*

N application affected root structural development (Table 3 and Figure 5). The cell number and cross-sectional area of the root elongation zone were higher under N treatment, and their differences were significant at $p < 0.01$ (Table 3). The effects of N on the size and shape of cells was probably due to enhanced enzyme synthesis and metabolic functions during growth (Ching et al., 1978).

Table 3. Effect of N application on cell number and cross-sectional area of elongation zone of progeny wheat roots

| Varieties | treatment | Cell number of elongation zone | cross-sectional area of elongation zone ($mm^2$) |
|---|---|---|---|
| Zheng 9203 | CK | 306A | 0.129A |
| | N | 364B | 0.159B |
| Yangmai 13 | CK | 323A | 0.119A |
| | N | 346B | 0.150B |

Values within each column marked with different upper case letters indicate a highly significant difference ($p < 0.01$).

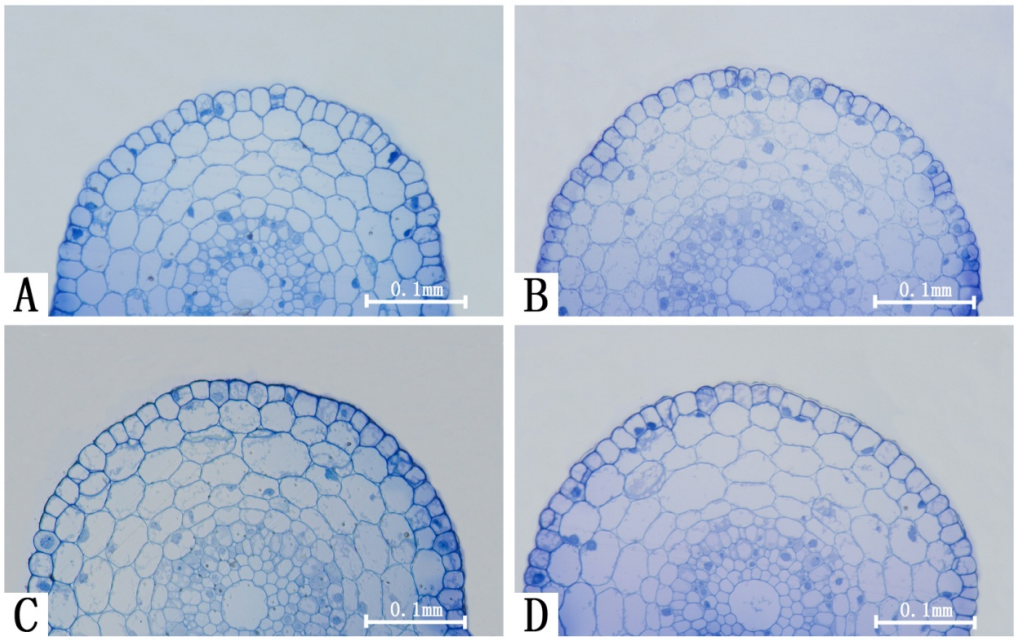


Figure 5. LM images of transverse sections of seedling root at 3 DPG in cv. Zheng 9023 in control (A), cv. Zheng 9023 with N treatment (B), cv. Yangmai 13 in control (C), cv. Yangmai 13 with N treatment (D)

### *3.7 Effect of N Treatment on Endosperm Starch Degradation*

The endosperm starch near the embryo began to degrade at 3 DPG and starch granules were almost completely hydrolyzed after 9 d. The rates of starch degradation were affected by N application in both cultivars (Figure 6; Figure 7). For cv. Zheng 9023, the consumption of endosperm starch under N treatment (Figures 7B and D) was slower and the groove between embryo and endosperm were smaller compared to controls (Figures 7A and C)- furthermore, hydrolysis of starch granules was also slower and the cell walls of endosperm were intact (Figure 6B). However, the results for cv. Yangmai 13 were in contrast: for N treatment the endosperm starch degraded more rapidly (Figures 7F and H) and grooves were bigger than in controls. In addition, many endosperm cell walls had decomposed or were decomposing and starch granules escaped from cells and disappeared (Figure 6D). Thus, the effects of N on endosperm starch degradation differed between the varieties.

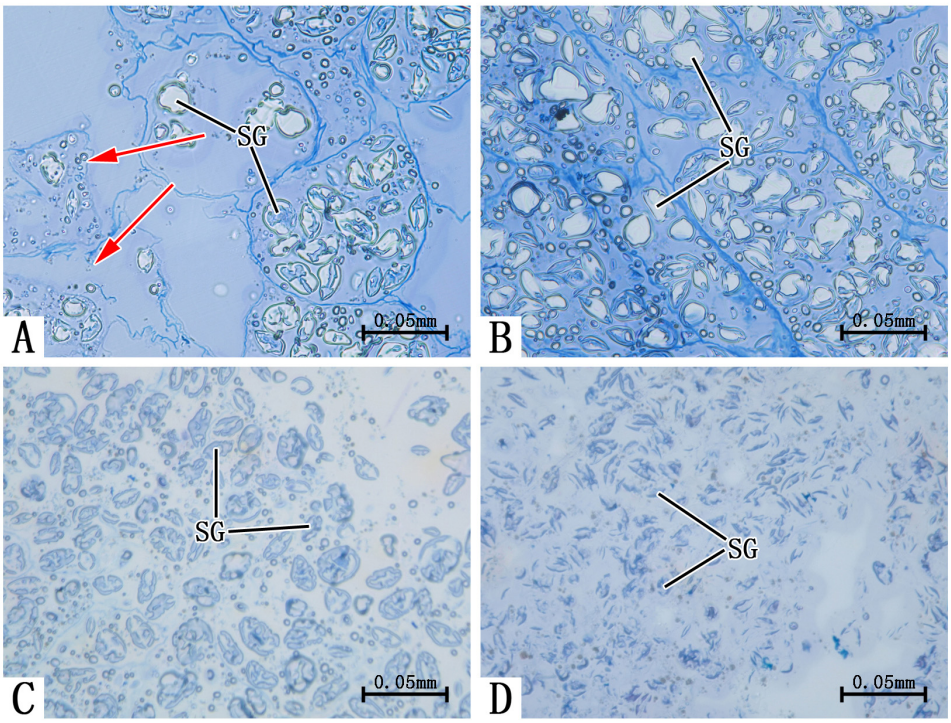


Figure 6. Cross sections of endosperm at 3 DPG under LM (× 400) in cv. Zheng 9023 in control (A), cv. Zheng 9023 with N treatment (B), cv. Yangmai 13 (C) in control, and cv. Yangmai 13 with N treatment (D). The red arrows indicate that cell walls were decomposing. The black bars indicate 0.05 mm. sg: starch granule

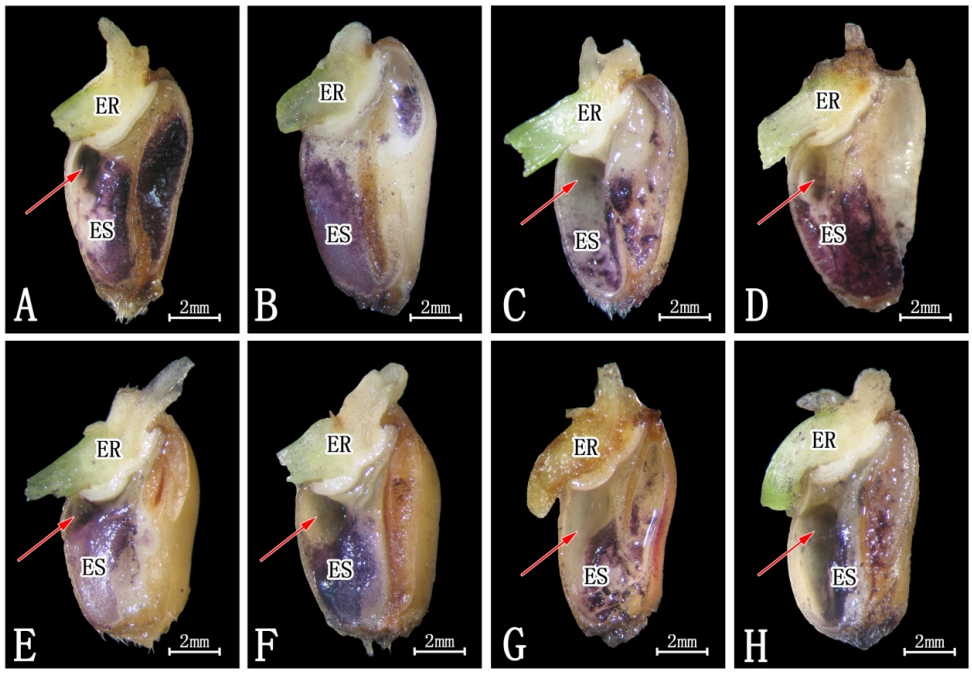


Figure 7. Vertical sections of germinating seeds under LM (×10). A, C: seeds of cv. Zheng 9023 in control at 3 and 6 DPG, respectively; B, D: seeds of cv. Zheng 9023 with N treatment at 3 and 6 DPG, respectively; E, G: seeds of cv. Yangmai 13 in control at 3 and 6 DPG, respectively; F, H: seeds of cv. Yangmai 13 with N treatment at 3 and 6 DPG, respectively. The red arrows indicate grooves of starch endosperm degradation. The blue-black indicates endosperm starch stained by $I_2$-KI. The white bars indicate 2 mm

## 4. Discussion

Impact of N on the progeny following wheat seed germination was studied by observing the physiological and morphologic germination indices. The results showed N treatment resulted in higher protein contents, higher chlorophyll contents, higher activities of SOD and dehydrogenase, greater cell numbers and cross-sectional areas of root, and a faster rate of seedling growth.

Results for germination percentage, germination rate and endosperm starch degradation under N treatment in the present study contrasted between cvs. Yangmai 13 and Zheng 9023. It is well known that the food reserve necessary for germination is stored in the endosperm, and that the endosperm starch is mainly responsible for the germination of seeds during their early stages. The starch contents were higher for cv. Yangmai 13, compared to cv. Zheng 9023, and so it could supply more materials for germination. In addition, starch degradation of cv. Yangmai 13 was faster, probably due to a faster transport rate of hydrolytic products from endosperm into embryo or the seedling axes in cv. Yangmai 13.

SOD activity is an important antioxidant defense in nearly all cells (Zhang et al., 2007; Dou et al., 2010). Mo et al. reported that rice seeds with high protein contents had high SOD activity during germination (Mo et al., 2004); a result in agreement with our findings. Moreover, we also found that cell number and cross-sectional area of roots increased under N treatment. Therefore, it appeared that improved root vigor and progeny quality of seedlings was affected by applying a certain amount of N at booting.

These experiments were done only in the laboratory, and the effects on seed germination and seedling growth in the field and their molecular mechanisms need further investigation.

**Acknowledgments**

The research was supported by the National Natural Science Foundation (31171482), Jiangsu Natural Science Foundation (BK2011445), Jiangsu Graduate Innovation Project (CXLX12-0910).

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