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Full Length Article

Effects of Low Temperature on Leaf Anatomy and Photosynthetic Performance in Different Genotypes of Wheat Following a Rice Crop

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Abstract

The effect of low temperature on leaf anatomy and photosynthetic performance of two wheat (*Triticum L.*) genotypes grown after rice (*Oryza sativa L.*) was investigated. After exposure to low temperature, the arrangement of mesophyll cells in *cv.* Zhengmai 9023 (poor cold tolerance) was more irregular than *cv.* Yannong 19, being a cold tolerant variety. Mesophyll cells shrank, and its vessels and sieve tubes ruptured at the tillering stage. Net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), and stomatal conductance (G_s) declined significantly in the low temperature treatment compared with control across both cultivars, but the decreased amplitude in P_n and G_s were greater for *cv.* Zhengmai 9023 than *cv.* Yannong 19. Compared with the control, initial fluorescence (*Fo*), non-photochemical quenching (NPQ), and the acyclic photosynthetic electron transfer rate of PSII (ETR) generally increased, but the maximum photochemical efficiency of PSII (F_v/F_m) and the photochemical quenching coefficient (*q*P) generally decreased in low temperature treatment. After the low temperature treatment, the increase in F_o parameter of *cv.* Zhengmai 9023 was greater than *cv.* Yannong 19. In contrast, the decreases in F_v/F_m, *q*P, and ETR were greater for *cv.* Zhengmai 9023 than *cv.* Yannong 19, but the increase in NPQ in Yannong 19 was greater than Zhengmai 9023. The results suggest that exposure to low temperatures at the tillering and stem elongation stages can significantly affect leaf anatomy and photosynthetic performance in wheat. Meanwhile, the photosynthetic apparatus was damaged only slightly and showed a high level of photosynthetic activity and a strong self-protection mechanism when the strong cold tolerance cultivars were chosen.

Keywords: Low temperature stress; Wheat; Photosynthetic performance; Leaf anatomical structure

Introduction

The total area in China under wheat cultivation after rice ranks first in the world and wheat is planted following the rice crop in both south and north China regions, mainly distributed in the Yangtze River basin. In recent years, with the frequent occurrence of extreme weather, cold injury at the seeding stage before winter, as well as cold damage at the stem elongation stage are the main environmental stresses in the area with wheat planted after harvesting rice. Spring varieties are the most common types of wheat planted after a rice crop and have poor cold tolerance. Additionally, early sowing or high seedling densities in late sowing can lead to fast growth before winter, resulting in thin and fragile wheat seedlings with reduced cold tolerance. When sudden cooling occurs before winter or continuous low temperatures in spring, wheat seedlings are likely to incur chilling damage, causing a series of ultra-structural, physiological and biochemical changes (Li *et al*., 2014).

When subjected to low temperature stress, plant cells and tissue structures may undergo changes. The critical low temperature would result in metabolic changes and functional disorder in plant cells (Lyons, 1973). Upon exposure to low temperatures, mesophyll cells in the phyllode of *Acacia melanoxylon* shrank and the intercellular spaces increased with decreasing temperature, due to apparent freezing of the bulliform cells (Ruan *et al.*, 2011). Increased intercellular space could prevent freezing inside cells, therefore, the relative tightness of the leaf tissue structure is considered to be associated with plant cold tolerance (Yu *et al*., 2010). Similarly, wheat varieties with strong cold tolerance under cold acclimation, the proportion of vacuoles in the tiller node cells was small, as was the intracellular frozen area, which reduced freezing damage to the cells (Fu *et al.*, 2010).

Photosynthetic intensity in many plant species declines after low temperature treatment (Toylar and Rowley, 1971). Under conditions of low temperature stress, the net photosynthetic rate (P_n) in most plants decreases significantly. After exposed to low temperature stress, the photosynthetic rate and Hill reaction activity in wheat seedling leaves decreased greatly (You *et al*., 2002). For

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varieties with strong cold tolerance, the intercellular CO_2 concentration (C_i) decreased and the leaf C_i decreased along with decreasing stomatal conductance (G_s) during exposure to low temperature. However, for the varieties with poor cold tolerance, C_i increased while G_s showed a decreasing trend (Zhu *et al.*, 2006; Fan *et al.*, 2009). After treatment, chlorophyll *a* and *b* contents in the wheat leaf were significantly decreased, the core components of PSI and PSII as well as the peripheral antenna, were subjected to a certain degree of damage; this affected the absorption and delivery of light energy, eventually resulting in decrease of photosynthetic intensity (You *et al*., 2002). The level of chlorophyll fluorescence in living plants reflects the utilization and dissipation of excitation energy by PSII, an effective probe of photosynthesis. The chlorophyll fluorescence of rice seedlings were significantly changed after subjected to cold treatment for different durations (Suzuki *et al.*, 2008). Rapid fluorescence dynamics could be used to screen wheat varieties for strong cold tolerance after *in vitro* freezing experiments on wheat leaves (Rapacz and Wozniczka, 2009). Wang *et al.* (2009) compared the chlorophyll fluorescence of different winter wheat varieties under cold acclimation and freezing and pointed out that chlorophyll fluorescence parameters could be used for the identification of varieties with different levels of cold tolerance in production. Under weak light, the actual photochemical efficiency (Φ_{PSII}) and photochemical quenching (*q*P) in cucumber leaves decreased with decreasing temperature (Li *et al*., 2008). After low temperature treatment at 3ºC, the F_v/F_m in mulberry leaves decreased significantly, and was related to structural and functional changes in the photosynthetic apparatus caused by cold stress (Xu and Sun, 2009; Gao *et al.*, 2013).

Many recent studies focusing the effects of low temperature stress on the physiological and biochemical aspects of wheat, but few reports describe the effects of low temperature stress on different wheat genotypes at different growth stages in the rice-wheat crop rotation system. In this study, we examined the effects of cold stress on wheat grown after a rice crop in the Jianghuai area. We selected two different wheat varieties of different genotypes (spring and semi-winter) for low-temperature treatments at the tillering and stem elongation stages. We investigated changes in the anatomical structure of the wheat leaf and the tillering node, as well as the photosynthetic performance of the functional leaf. This work confirmed the physiological basis of the effects of low temperature on the growth and development of wheat from overwintering to the stem elongation stage.

Materials and Methods

Plants

The experimental material was two wheat *cv.* Zhengmai 9023 (a spring wheat variety, bred at the Henan Academy of Agricultural Sciences) and Yannong 19 (a semi-winter variety, bred at the Yantai Agricultural Sciences and Technology Institute in Shandong).

Experiment Design

The experiment was carried out at Da Yang Dian test base, Anhui Agricultural University, Hefei, Anhui (north latitude of 31°52′, east longitude of 117°17′) from November 2011 to June 2013. The previous crop was rice, and the test varieties were sown on October 26, 2011 and November 8, 2012. Seedlings were grown in pots with a diameter of 30 cm and a height of 30 cm, 20 pots per variety, 15 plants per pot and the pots were buried in the experimental field. Three replicates for each variety were assigned randomly in the field. Field management was performed according to the requirements of high-yield cultivation.

The potted wheat seedlings were transferred indoors at the tillering stage (December 28, 2011), moved into a -10ºC refrigerator at 20:00 and then moved outside 12 h later; the treatment was for two days. The potted wheat plants were taken indoors at the elongation stage (March 4, 2012 and March 8, 2013), transferred to a 0ºC refrigerator at 20:00, and moved outside at 08:00 the next day; the treatment continued for three days. At every stage, three pots of each variety were treated, and the untreated plants of the same varieties were used as controls.

Microscopic Observations, Measurements, and Methods

(1) We selected the penultimate leaf on the main stem and the tillering node before and after low temperature treatment at the tillering and stem elongation stages.
(2) Fixation: leaves were cut into small pieces of 5 mm × 3 mm, and the tillering node was cut into pieces of 2 mm × 2 mm. A needle cylinder was used for vaccuming, and the tissue pieces were immediately transferred to FAA fixing solution (consisting of 5 ml of 38% formaldehyde, 5 mL of glacial acetic acid, 90 mL of 95% ethanol).
(3) Pre-staining: the fixed materials were immersed in the following solutions in series: 70% alcohol, 50% alcohol, 1% sarranine aqueous solution, distilled water, and 50% alcohol. Treatment was for 1 h in all alcohol solutions and distilled water and for 10 h in 1% sarranine aqueous solution.
(4) Dehydration: the pre-stained materials were immersed in the following solutions in series: 70% alcohol, 85% alcohol, 95% alcohol, absolute alcohol, absolute alcohol. Treatment was for >2 h in 70% alcohol, and 0.75 ~ 2 h for the other alcohol solutions.
(5) Clearing: the dehydrated materials were immersed in 50% absolute alcohol: 50% xylene, absolute xylene and absolute xylene in series, each for 1.5 h.
(6) Waxing: after clearing, the materials were transferred to a mixture of xylene and broken wax for 1–2 h, then transferred into molten pure wax for 3 h (3 times); waxing

was carried out in an incubator at 2ºC above the melting point of paraffin.

(7) Embedding and sectioning: molten wax was poured into an embedding box and the waxed tissue pieces were then transferred into the wax. The paraffin block was removed from the mold after it had completely solidified. Using a blade, the paraffin block was cut into square or rectangular pieces, which were then cut into sections with a thickness of 4–7 μm.

(8) Dewaxing, staining and mounting: the sections were immersed in the following solutions in series: absolute xylene (1–15 min), absolute xylene (1–15 min), 50% absolute alcohol: 50% xylene (1–5 min), absolute alcohol (1–2 min), 95% alcohol (1–2 min), 85% alcohol (1–2 min), 70% alcohol (1–2 min), 50% alcohol (1–2 min), distilled water (1–3 min), 1% sarranine aqueous solution (2–12 h), distilled water (1–3 min), 50% alcohol (1–2 min), 70% alcohol (1–2 min), 85% alcohol (1–2 min), 95% ethanol (1–2 min), 0.5% fast green alcohol (15–60 s), 95% alcohol (1–2 min, twice), absolute alcohol (1–2 min, twice), 50% absolute alcohol:50% xylene (1–5 min), absolute xylene (1–2 min, twice), and finally gum for mounting.

Measurement of Photosynthetic Performance in Wheat Leaves

Measurements were performed with the LI-6400 XT portable photosynthesis measuring system (manufactured by LI-COR company in USA) at 11:00 am on the treatment day: the intensity of the built-in light source was set to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the P_n, G_s, and C_i of the penultimate leaf of the wheat main stem were determined before and after low temperature treatment.

Chlorophyll fluorescence was measure with the IMAGING-PAM (MINI) chlorophyll fluorescence spectrometer (manufactured by WALZ company in Germany) at 13:00 on the treatment day: the F_o and F_v/F_m of the wheat main stem penultimate leaf were determined after dark adaptation for 20 min before and after low temperature treatment, and the *q*P, NPQ, and ETR were determined with actinic light (800 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Microscopic Observations, Measurements, and Methods: Micrographic observation: the sections were examined and photographed using an Olympus BXB51\BXB52 microscope. Image - Pro Express 5.1 Image analysis software was used for image processing.

All data were statistically analyzed with statistical software SPSS (version 16.0) and Microsoft Excel.

Results

Effects on Wheat Leaf Anatomy

The epidermal and mesophyll cells can clearly be seen in the wheat leaf not subjected to low temperature treatment, and the upper and lower epidermal cells are arranged closely (Figs. 1, 2). After low temperature treatment, two wheat varieties (-10ºC at the tillering stage and 0ºC at elongation), the mesophyll cells shrank with increasing intercellular spaces. Zhengmai 9023 treated with -10ºC at the tillering stage was the worst (Fig. 1b), and the degree of shrinkage of the mesophyll cells in Zhengmai 9023 (Fig. 1b, 2b) was more obvious than Yannong 19 (Fig. 1d, 2d). The arrangement of the cells was also less uniform than in Yannong 19.

Effects on Vessels and Sieve Tubes in the Wheat Tillering Node

Vessels and sieve tubes are the vascular elements responsible for the transport of water, inorganic salts, and organic nutrients in the xylem and phloem, respectively, in plants. When exposed to low temperatures for an extended period of time, the freeze volume in vessels and sieve tubes of the stem and leaf increased, resulting in rupture of the vessels and sieve tubes. The vessels and sieve tubes of the Zhengmai 9023 tillering node were ruptured after treatment with low temperature (-10ºC at the tillering stage) (Fig. 3).

Effects on the Photosynthetic Performance

After exposing to low temperature stress at the tillering and stem elongation stages, the P_n of the wheat leaf decreased significantly. C_i and G_s also showed a decreasing trend (Table 1). This trend was similar in the two-year effects; the differences before and after treatment were significant at 1% or 5% probability level. In the 2011–2012, the P_n and C_i for Zhengmai 9023 were higher than Yannong 19, while the G_s was lower prior to low temperature treatment both at the tillering and stem elongation stages. After treatment at -10ºC and 0ºC, the P_n and G_s, respectively, for Yannong 19 were higher than Zhengmai 9023 and C_i was lower. After treatment, the relative declines in P_n and G_s for Zhengmai 9023 were greater than Yannong 19: after treatment at -10ºC, the declines in P_n and G_s for Zhengmai 9023 were 79.76% and 75.50% respectively, while after treatment at 0ºC, P_n and G_s declined by 45.45% and 53.45%, respectively. For Yannong 19, the declines in P_n and G_s following treatment at -10ºC were 67.75% and 67.63% respectively, while at 0ºC resulted in reductions in P_n and G_s of 34.17% and 39.78%, respectively. The decrease in C_i for Zhengmai 9023 was less than Yannong 19; after treatment at -10ºC and, with decline of 4.74% and 26.59%, respectively, and the C_i for Zhengmai 9023 and Yannong 19 declined by 6.23% and 14.06%, respectively after treatment at 0ºC. In the 2013 trials, the P_n and G_s for Yannong 19 were higher than Zhengmai 9023, while C_i was lower prior to low temperature treatment at the stem elongation stage. However, after treatment at 0ºC, the P_n, G_s, and C_i for Yannong 19 were all higher than Zhengmai 9023, which was not completely consistent with the performance in the 2012 trials after the 0ºC treatment at the stem elongation

stage. The relative decrease in all photosynthetic parameters for Zhengmai 9023 were greater after treatment at 0ºC; the declines in P_n, G_s, and C_i for Zhengmai 9023 were 42.34%, 48.09%, and 47.73% respectively, and for Yannong 19 38.52%, 27.06%, and 32.87%.

Effects on Chlorophyll Fluorescence Characteristics

The changes in the chlorophyll fluorescence parameters in wheat leaves exposed to low temperature stress at the tillering and stem elongation stages were not consistent (Table 2). In the 2011–2012, *Fo* showed an increasing trend after low temperature treatment, and the difference observed in Yannong 19 after treatment at 0ºC during the stem elongation stage was significant (P<0.05) while, the other differences between treated and untreated were not.

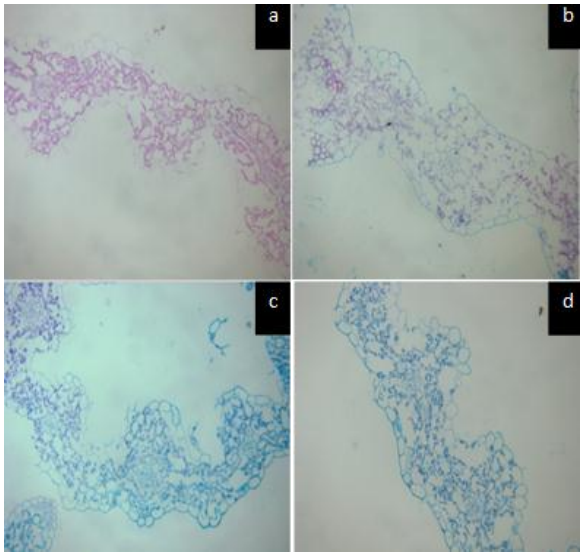

Fig. 1: Effects of low temperature stress on the anatomical structure of the penultimate leaf of two wheat genotypes at the tillering stage (×20). (a) Zhengmai 9023. (b) Zhengmai 9023 treated with -10ºC. (c) Yannong 19. (d) Yannong 19 treated with -10ºC

The F_v/F_m showed a decreasing trend after low temperature treatment; the differences in the two varieties before and after treatment were all significant after treatment at -10ºC at the tillering stage, but were not significant after treatment at 0ºC at the stem elongation stage. The *q*P showed a decreasing trend after low temperature treatment, and the differences were significant for treatment at -10ºC at tillering and 0ºC at stem elongation stages. The NPQ showed an increasing trend after low temperature treatment except for the significant decrease observed in Zhengmai 9023 after treatment at tillering stage. The ETR showed a decreasing trend after low temperature treatment and the differences between the two varieties before and after treatment was significant at the tillering and stem elongation stages.

Combining the changes in the chlorophyll fluorescence parameters before and after low temperature treatment at the two stages, we found that after low temperature treatment, the increase in F_o for Zhengmai 9023 was higher than Yannong 19, the decreases in F_v/F_m, *q*P, and ETR were all higher for Zhengmai 9023 than Yannong 19, and the increase in NPQ in Yannong 19 was higher than Zhengmai 9023 except that the NPQ in Zhengmai 9023 showed a decreasing trend at tillering stage.

For the two varieties before and after treatment at 0ºC, the difference in F_o was significant, and differences in the other chlorophyll fluorescence parameters were highly significant (data not shown). After treatment, the F_o for Yannong 19 decreased, but it increased somewhat in Zhengmai 9023; the F_v/F_m increased in Yannong 19 and declined somewhat in Zhengmai 9023; the *q*P declined in both varieties, while the NPQ increased (Fig. 4). After comparing the two varieties, before treatment, no significant difference was found for *q*P between the two varieties, other chlorophyll fluorescence parameters for Zhengmai 9023 were significant or highly significant compared to Yannong 19. After treatment at 0ºC, the F_o and NPQ for Zhengmai 9023 were all significantly higher than Yannong 19, and F_v/F_m and *q*P were significantly lower than Yannong 19 (data not shown).

Table 1: Effects of low temperature stress on photosynthetic parameters in two different wheat genotypes at the tillering and stem elongation stages

Year	Stage	Variety	Treatment	P_n(μ mol·m^{-2}·s^{-1})	G_s(mol·m^{-2}·s^{-1})	C_i (μ mol·mol^{-1})
2011-2012	Tillering stage	Yannong 19	control	13.55 ^{Aa}±0.16	0.17 ^{Aa}±0.02	225.27 ^{Aa}±2.54
			-10℃	4.37 ^{Bb}±0.05	0.05 ^{Bb}±0.00	165.38 ^{Bb}±3.41
		Zhengmai 9023	control	17.15 ^{Aa}±0.15	0.20 ^{Aa}±0.01	179.90 ^{Aa}±1.39
			-10℃	3.47 ^{Bb}±0.04	0.05 ^{Bb}±0.00	171.37 ^{Ab}±1.40
	Stem elongation stage	Yannong 19	control	16.02 ^{Aa}±0.10	0.17 ^{Aa}±0.00	174.90 ^{Aa}±2.53
			0℃	10.55 ^{Bb}±0.05	0.10 ^{Bb}±0.01	150.32 ^{Ab}±4.30
		Zhengmai 9023	control	17.35 ^{Aa}±0.15	0.18 ^{Aa}±0.00	166.51 ^{Aa}±1.98
			0℃	9.46 ^{Bb}±0.15	0.08 ^{Bb}±0.00	156.13 ^{Ab}±4.81
2012-2013	Stem longation stage	Yannong 19	control	10.38 ^{Aa}±0.49	0.09 ^{Aa}±0.00	199.59 ^{Aa}±8.85
			0℃	6.38 ^{Bb}±0.38	0.06 ^{Aa}±0.01	133.99 ^{Bb}±3.60
		Zhengmai 9023	control	8.92 ^{Aa}±0.19	0.08 ^{Aa}±0.01	248.46 ^{Aa}±5.36
			0℃	5.14 ^{Bb}±0.40	0.04 ^{Bb}±0.00	129.87 ^{Bb}±7.60

P_n net photosynthetic rate, G_s stomatal conductance, C_i intercellular CO_2 concentration

Values followed by the same lower case letter do not significantly different between control without low temperature stress and exposed to low temperature at 0.05 levels, and the same capital letter means do not significantly different at 0.01 levels

Table 2: Effects of low temperature stress on chlorophyll fluorescence parameters in two different wheat genotypes at the tillering and stem elongation stages

Stage	Variety	Treatment	F_o	F_v/F_m	qP	NPQ	ETR
Tillering stage	Yannong 19	control	143.67 ^{Bb}±1.67	0.81 ^{Aa}±0.00	0.32 ^{Aa}±0.01	2.29 ^{Ab}±0.05	48.24 ^{Aa}±1.07
		-10℃	155.97 ^{Aa}±2.63	0.80 ^{Ab}±0.00	0.27 ^{Ab}±0.00	3.00 ^{Aa}±0.05	37.06 ^{Bb}±0.66
	Zhengmai 9023	control	145.10 ^{Bb}±1.03	0.82 ^{Aa}±0.00	0.27 ^{Aa}±0.01	2.70 ^{Aa}±0.02	48.48 ^{Aa}±0.33
		-10℃	159.83 ^{Aa}±1.93	0.77 ^{Ab}±0.00	0.22 ^{Ab}±0.00	1.90 ^{Bb}±0.01	27.45 ^{Bb}±0.57
Stem elongation stage	Yannong 19	control	151.96 ^{Bb}±4.95	0.84 ^{Aa}±0.00	0.43 ^{Aa}±0.00	2.45 ^{Aa}±0.03	98.70 ^{Aa}±1.68
		0℃	157.22 ^{Aa}±5.71	0.84 ^{Aa}±0.00	0.39 ^{Bb}±0.01	2.59 ^{Aa}±0.07	87.26 ^{Ab}±3.33
	Zhengmai 9023	control	194.05 ^{Ab}±1.96	0.84 ^{Aa}±0.00	0.45 ^{Aa}±0.01	2.42 ^{Ab}±0.05	97.43 ^{Aa}±1.68
		0℃	202.19 ^{Aa}±5.78	0.83 ^{Aa}±0.00	0.37 ^{Bb}±0.01	2.55 ^{Aa}±0.04	83.03 ^{Ab}±2.68

F_o initial fluorescence, F_v/F_m maximum photochemical efficiency of PSII, qP photochemical quenching coefficient, NPQ non-photochemical quenching, ETR acyclic photosynthetic electron transfer rate of PSII

Values followed by the same lower case letter do not significantly different between control without low temperature stress and exposed to low temperature at 0.05 levels, and the same capital letter means do not significantly different at 0.01 levels

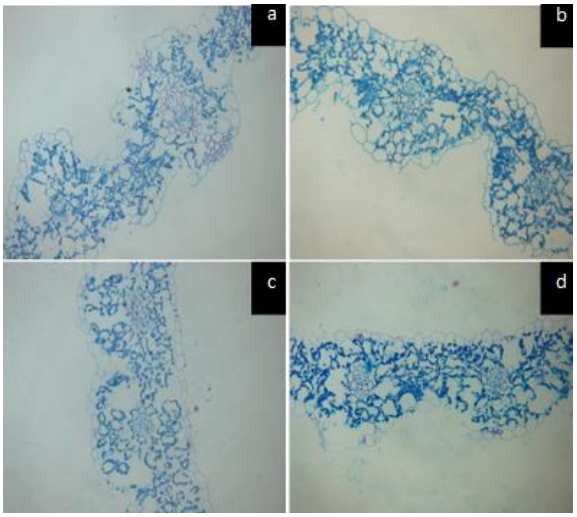

Fig. 2: Effects of low temperature stress on the anatomical structure of the penultimate leaf of two wheat genotypes at the stem elongation stage (×20). a Zhengmai 9023. b Zhengmai 9023 treated with 0ºC. c Yannong 19. d Yannong 19 treated with 0ºC

Discussion

Under low temperature stress, the arrangement of mesophyll cells in wheat line with poor cold tolerance had been reported more irregular than cultivar with strong cold tolerance (Arora and Palta, 1991). For wheat with strong cold tolerance, the mesophyll cells were small and arranged closely, with many layers advantageous to the arrangement of chloroplast on the mesophyll cell surface (Miao *et al*., 2006). In our experiment, after low temperature treatment at the tillering and stem elongation stages, mesophyll cells in two wheat varieties showed shrinkage to varying degrees, with enlarged intercellular spaces, which was similar to our previous reports on cell structure in tiller node under low temperature stress (Zhang *et al*., 2012). After low temperature treatment at the tillering stage, the arrangement of mesophyll cells in Zhengmai 9023 was more irregular than Yannong 19; while at the stem elongation stage, shrinkage of mesophyll cells in Zhengmai 9023 was more pronounced than Yannong 19, indicating that Zhengmai 9023 was more sensitive to the damaging effects of low temperature. In addition, after exposure to low temperature stress, the internal water in vessels and sieve tubes in the Zhengmai 9023 tillering node froze, resulting in their rupture. This in turn prevented the transport of water and organic compounds, which negatively affect the subsequent growth and development of the plants.

Low temperatures inhibit photosynthesis through stomatal closure (Bertamini *et al*., 2007). In industrial chicory, cold stress affect the activity of enzymes involved in CO_2 fixation or the photosynthetic utilization of CO_2 (Devacht *et al*., 2009). In corn under cold stress, the CO_2 fixation rate was affected initially, and the activity of the photosynthetic electron transport chain in the thylakoidal membranes further reduced (Holá *et al*., 2003). Under low temperature stress, wheat stomates are partially closed, leading to blocked transport of CO_2 to the chloroplasts, as well as a decrease in C_i and P_n. But there was another kind of circumstance, low temperature stress influence the stomatal closure, meanwhile destroy the photosynthetic apparatus, finally, resulting in decline of the assimilatory power of CO_2, and the excessive accumulation of CO_2 led to an increase of C_i and decrease of P_n (Farquhar and Sharkey, 1982; Sun *et al*., 2006). In our experiments, for the two wheat varieties exposed to low temperatures at the tillering and stem elongation stages, the leaf P_n, C_i, and G_s were decreased significantly, suggesting that the trends in changes in the photosynthetic rate and intercellular CO_2 concentration were the same after exposure to low temperature stress, and the decline in the photosynthetic rate was mainly dominated by stomatal limitation. The decreases observed in P_n and G_s for Zhengmai 9023, a spring variety with poor cold tolerance, was higher than Yannong 19, a semi-winter variety with strong cold tolerance, indicating that the degree of inhibition in photosynthetic activity was higher for varieties with poor cold resistance. It was worthy to note that the relative decline in C_i was not consistent with the trials in two years; the C_i was greater for Yannong 19 in 2011–2012, but higher for Zhengmai 9023 in 2013, which could be due to differences in ambient temperature or

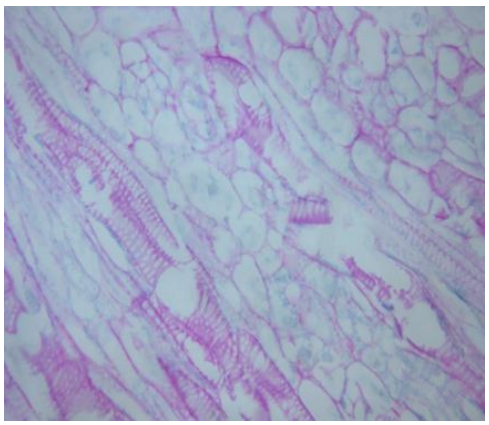

Fig. 3: Effects of low temperature stress on vessels and sieve tube in the tiller node of Zhengmai 9023 at the tillering stage (×40)

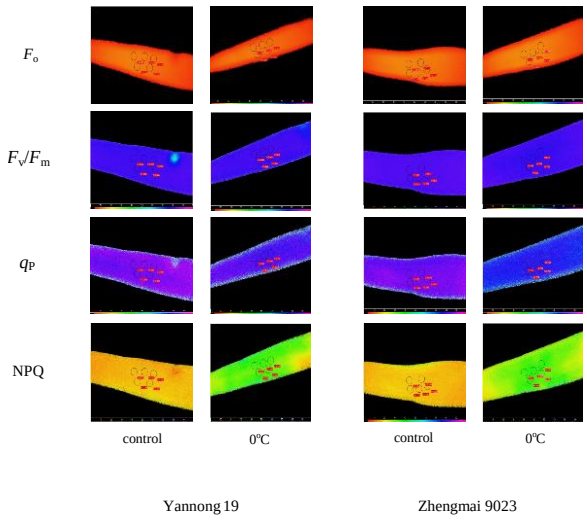

Fig. 4: Imaging of fluorescence parameters before and after low temperature stress in wheat at the stem elongation stage

environmental conditions between the two years as well as growth conditions between the two wheat varieties. This could have resulted in variations in the intercellular CO_2 absorption and utilization due to the decreased size of the stomatal aperture after exposed to low temperature stress (Farquha and Sharkey, 1982).

Dissipation of non-photochemical energy is likely to cause a decrease in F_o, which could be increased by damage to the photosynthetic apparatus (Zhang, 1999). A slight increase in F_o was due to the partially reversible reduction of PSII light quanta, and a greater increase may be due to the irreversible separation of PSII light capture complex (Briantais *et al.*, 1996). When treated with low temperatures, F_o showed an increasing trend, indicating that the photosynthetic apparatus in leaves of the both cultivars had been damaged. The increase in F_o for Zhengmai 9023 was significantly higher than Yannong 19, indicating that its photosynthetic apparatus had been seriously damaged, and was more obvious at the stem elongation stage. This suggests that low temperature stress at elongation had a much more significant effect on Zhengmai 9023. Values for F_v/F_m generally range between 0.74–0.85 (Lichtenthaler *et al.*, 2005), and this ratio is regarded as a sensitive index of photosynthetic performance. The F_v/F_m is also a sensitive index of plant photoinhibition. The lower the value, the more severely the plants are suffering from stress (Demmig and Björkman, 1987; Maxwell and Johnson, 2000). A decrease in F_v/F_m could be the result of photochemical damage in the PSII reaction center, but could also be the result of photoprotection, which is the dissipation of non-photochemical energy (Demmig and Björkman, 1987). The F_v/F_m is having been used to identify cold tolerance in a variety of plants (Ying *et al.*, 2000; Perks *et al.*, 2004). In our experiments, F_v/F_m showed a decreasing trend after low temperature treatment at both tillering and stem elongation stages, and was significantly different before and after treatment, indicating that PSII photochemical efficiency in the tested wheat leaves decreased after exposure to low temperature stress. After low temperature treatment, F_v/F_m decrease was higher for Yannong 19, with the relative decrease greater than Zhengmai 9023, suggesting former good resistance to low temperatures. The *q*P reflects the photosynthetic activity, while the NPQ reflects the photoprotection ability (Maxwell and Johnson, 2000; Kramer *et al.*, 2004). Alfalfa exposed to mild low temperature stress, the NPQ increased (Chen *et al.*, 2011). With decreasing temperature and extended time, NPQ in the maize seedling leaf decreased (Yang *et al.*, 2012). After exposure to low temperature stress at the tillering and stem elongation stages, the decrease in *q*P and increase in NPQ showed increase in the dissipation of non-photochemical energy, indicating that photoprotection was activated to avoid damage caused by excess light. The *q*P and NPQ in Zhengmai 9023 at tillering both decreased, suggesting that severe damage had been caused by excess light at that time. The *q*P and NPQ in Yannong 19 were much higher after cold treatment, suggesting that this variety had high photosynthetic activity and an effective self-protection mechanism following low temperature treatment. The ETR approximates the pumping rate of electrons through the photosynthetic chain and is closely related to the photosynthetic activity (Ralph and Gademann, 2005). After exposing to low temperature stress at the tillering and stem elongation stages, the ETR of the wheat leaf showed a decreasing trend; the ETR in Yannong 19 was comparatively high, while the ETR in Zhengmai 9023 showed an obvious decrease after low temperature treatment.

Conclusion

After exposed to low temperature stress at the tillering and stem elongation stages, the anatomical structure and

photosynthetic performance of the wheat leaf were significantly affected. Photosynthetic activity was inhibited to varying degrees in two wheat variety. Yannong 19 was damaged only slightly and displayed a comparatively high level of photosynthetic activity. Zhengmai 9023 (with poor cold tolerance) suffered from more serious effect whether at the tillering or stem elongation stage, and it was more sensitive at the stem elongation stage. Our findings provided a theoretical basis for the choice of wheat varieties in rice-wheat rotation system.

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