Influence of uniconazole and plant density on nitrogen content and grain quality in winter wheat in South China

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ABSTRACT

Superior protein quality and consistent processing quality is needed for winter wheat marketing in South China. It has been shown that uniconazole concentration and plant density are certainly related to crop growth. An experiment was conducted to investigate the effects of uniconazole concentration and plant density on nitrogen content and grain quality in winter wheat (*Triticum aestivum* L.). Trials were managed to provide three levels of density (90 × 10^4, 180 × 10^4, and 270 × 10^4 per ha) over plots receiving four levels of uniconazole concentrations (0, 10, 20, and 40 mg/kg) which were applied to seeds before sowing. The results revealed that the contents of N accumulated in ear, stem, and leaf were higher in uniconazole concentrations than that in control, and the effect of uniconazole on main stem was bigger than that on tillers. The grain protein was significantly (LSD, $P < 0.05$) higher in uniconazole concentrations than that in control. Uniconazole at 20 mg/kg was the most favorable for improving grain protein and protein fractions. Application of uniconazole concentrations also significantly (LSD, $P < 0.05$) increased WGC (wet gluten content) and SDS (sedimentation volumes), prolonged DDT (dough development time) and DST (dough stable time), and improved WA (water absorption), increased VV (valorimeter value), and subsequently improved the processing quality of wheat grains. These results suggest that a combination of uniconazole concentration and plant density should be applied in South China.

Keywords: grain protein; N distribution; protein composition; processing quality; uniconazole; density; winter wheat

Increasing demand for superior protein quality wheat in Asian markets creates both challenges and opportunities for Chinese producers. The challenge is to produce consistently and deliver grain with qualities that can satisfy both bread and noodle applications. The main breeding strategy for improved grain protein was to select new varieties for high grain protein concentration (Cregan and van Berkum 1984), although work was also done at the physiological level (Wardlaw and Moncur 1976, Taek et al. 1988). North China is considered ideal for production of higher protein wheat, as the region has a relatively short grain fill period and high heat stress during grain filling. Sichuan Basin, in contrast, has been block by lack of cultivars, changing disease pressures and unusual weather patterns which were ideal for producing middle or low protein grain. However, due to decreasing demand and prices for low protein grain wheat and increasing international competition, growers are looking for high protein grain wheat to better fill the needs of the Asian market. This will be possible if management strategies are developed that could minimize variability in grain quality, highly variable precipitation, and the wide range of management practices used for wheat production in the Sichuan Basin.

Uniconazole [(E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-l-yl)-1-penten-3-ol] is a new plant growth retardant in the triazole family. It inhibits gibberellin biosynthesis within the plant (Izumi et al. 1984, Zhou and Leul 1999), reduces the concentration of endogenous indole-3-acetic acid, and increases the concentration of zeatin, ABA and ethylene (Izumi et al. 1988, Zhou and Leul 1999). Foliar application of uniconazole has been shown to retard leaf elongation, improve tiller number and root growth. Uniconazole applied as a foliar spray at the three-leaf stage improved plant growth, including plant height, leaf size and

number, leaf area per plant and increased seed and oil yields of winter rape compared to untreated plants (Leul and Zhou 1998). It was also shown to enhance plant photosynthetic rate, soluble protein, and total sugar concentrations (Yang et al. 2005a, Yang et al. 2005b). According to Gandee et al. (1997), Sekimoto et al. (1995) and Zhang et al. (2001), certain interactions existed between uniconazole and N fertilizer, which affected plant growth and yield formation. Our previous study also showed some interactions between uniconazole and different cultivars, sowing dates, planting densities, and N application levels which affected yield formation and enhancement (Yang et al. 2004). However, the effect of uniconazole on grain proteins is uncertain and available studies have not been reported.

As a result, this study discussed from N distribution viewpoint, was conducted to investigate variability in flour protein content and protein quality in relation to influences and interactions of uniconazole concentrations and density management in the Sichuan Basin.

MATERIAL AND METHODS

Plant material and treatments. The current research was conducted on a clay loam soil at the experimental farm of Sichuan Agricultural University (Yaan, 29.59°N, 102.62°E) during the 2000–2001 seasons, and was based on preliminary results with uniconazole concentration (Liang and Yang 1998, Yang et al. 2004) during the 1996–2000 seasons. A winter wheat cultivar Mianyang 26 was sown on October 30, 2000 on an untilled paddy field. The plots were 14 m^2 and laid out in a randomized complete block design with three replicates. A 5% water-dispersible powder of uniconazole (high effect triazole) was provided by the Jianhu Chemical Factory, Jiangsu Province. Trials were managed to provide three levels of densities (90×10^4, 180×10^4, and 270×10^4 per ha) over plots, D1, D2, and D3 are the code for the three densities, respectively; and before sowing, 10, 20, and 40 mg/kg uniconazole [(E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-l-yl)-1-penten-3-ol] (distilled water as control) was applied to seed. The concentration of uniconazole was selected according to our previous investigations (Liang and Yang 1998, Yang et al. 2004). Urea, super phosphate, and potash chloride were applied before sowing at the rates of 390, 300, and 150 kg/ha, respectively. The experimental treatments consisted of 12 combinations of density levels and uniconazole concentrations. The various coded treatments are detailed in Table 1.

N content of main stem and tillers at mature stage. At mature stage, 15 plant samples were taken from each plot for determining N content of main stem and tillers. At laboratory, samples were divided into stem (including leaf sheaths encircling the stem), leaf and ear (including husks and awns), main stem and tillers, weighing after drying for 0.5 h at 105°C and 48 h at 85°C. Dried plant tissues material was milled to pass through a 0.42 mm screen, and 100 mg sub-samples were digested for total N analysis by a Kjeldahl method which included 0.5% selenium as catalyst and salicylic acid to reduce nitrate-N (Eastin 1978).

Yield and yield components. At harvesting, 15 plants per plot were selected randomly. Yield components were measured from each sample, including ears per plant, grains per ear, and one thousand grain weight.

Protein content and protein composition. Grain was collected from three replications of each treatment. Seed samples of 1 kg derived from the bulk of the three replications of each treatment were used for milling and subsequent flour analyses. Wheat grains were milled on a 300 g Brabender Farinograph (Brabender OHG, Duisburg, Germany) following

Table 1. Treatments for density and uniconazole concentrations

Experimental factors	Code for treatments											
	D1B0	D1B2	D1B3	D1B4	D2B0	D2B2	D2B3	D2B4	D3B0	D3B2	D3B3	D3B4
Density (10^4/ha)	90	90	90	90	180	180	180	180	270	270	270	270
Uniconazole concentration (mg/kg)	0	10	20	40	0	10	20	40	0	10	20	40

D1, D2, D3 – density levels of 90×10^4, 180×10^4, and 270×10^4 per ha respectively; B0, B2, B3, B4 – 0, 10, 20, and 40 mg/kg uniconazole applied to seed, respectively

the procedure recommended by the manufacturer. Grain protein content was measured with a LECO FP-528 Nitrogen/Protein Determinator and calculated as: 5.7 × N% in grain dry matter. The protein fractions gliadin, glutenin, albumin, and globulin, were sequentially extracted from whole meal flour (Triboy et al. 2003). One sequential extraction and N concentration analysis was performed for each of the three independent replicates.

Physical dough tests. Mixograph curves (National Manufacturing Division, TMCO, Lincoln, NE, USA) were obtained with a 2 g computerized direct drive mixograph equipped with a water-jacketed bowl maintained at 25°C and 88 rpm. Mixograph parameters were recorded using Mixsmart software supplied with the instrument as described by Sapirstein et al. (2007). SDS sedimentation assays analyses were conducted as measures of protein quality and dough rheological properties (AACC Ref. Methods). Selected responses were: wet gluten content (WGC), water absorption (WA), dough development time (DDT), dough stability time (DST), mixing tolerance index (MTI), dough breakdown time (DBT), degree of softening (DS), valorimeter value (VV).

Statistical analysis. The experimental data were subjected to analysis of variance (ANOVA) at a significance level of $P = 0.05$ to determine if significant differences existed among means of the different treatments. Multiple comparisons were conducted for significant effects with the least significant difference (LSD) judged at $P < 0.05$.

RESULTS

N distribution in main stem and tillers at mature stage. At mature stage, the pattern of N distribution within the plant had an important impact on grain protein content. Until anthesis all plant parts were net importers of N (Oscarson et al. 1995) and both N concentration and N content increased with total dry weight. At mature stage, the total vegetative biomass continued to increase, and even though the N content of all above ground parts was decreasing, the weight and N content of ear continued to increase.

As shown in Figure 1, the content of N in stem and leaf decreased with increasing density, and it had no significant (LSD, $P < 0.05$) influence on N content in ear. However, the content of N in ear, stem, and leaf was changed with uniconazole applications, among which B3 was the highest; there was no significant (LSD, $P < 0.05$) difference between B2 and B4.

Generally, the changes of N content in vegetative tissues showed that the content of N remaining in the vegetative tissues was low at final harvest. Uniconazole was in favor of tillers-ear at low density D1, which provided plenty alimentation; otherwise, it was in favor of main stem-ear at higher density D2 and D3, which provided relatively less alimentation. All which indicated that uniconazole had effects on harmonizing N distribution in stem, leaf and ear.

Grain yield. As Table 2 indicates, the highest uniconazole concentration (B4) did not significantly increase grain yield, but this result was expected; as previously explained, the B3 treatment was estimated to be optimal for maximum yield. Mean grain yield over three densities in B4 treatment ranged from 5009.42 to 5260.46 kg/ha, which is comparable to that for B3 treatment ranging from 5446.91 to 5682.45 kg/ha. There were significant (LSD, $P < 0.05$) differences in grain yield between low and high uniconazole concentrations, as the uniconazole concentration changed grain yield at all densities, among which B3 gave the best results. Compared with control, the B3 treatment increased average grain yield by 22.59%.

Table 2. Effect of uniconazole and plant density on grain yield (kg/ha)

Treatment	B0	B2	B3	B4	Average
D1	4390.16	5088.91	5446.91	5123.80	5009.42^{c}
D2	4505.36	5125.16	5581.74	5163.23	5175.26^{b}
D3	4731.44	5326.09	5682.45	5318.49	5260.46^{a}
Average	4548.80^{c}	5182.57^{b}	5576.15^{a}	5206.93^{b}	

Each result is the average of three repetitions. Values followed by the same letter in the same column are not significantly different. Different small letters indicate significance at the 0.05 level. D1, D2, D3 – density levels of 90×10^4, 180×10^4, and 270×10^4 per ha, respectively; B0, B2, B3, B4 – 0, 10, 20, and 40 mg/kg uniconazole applied to seed, respectively

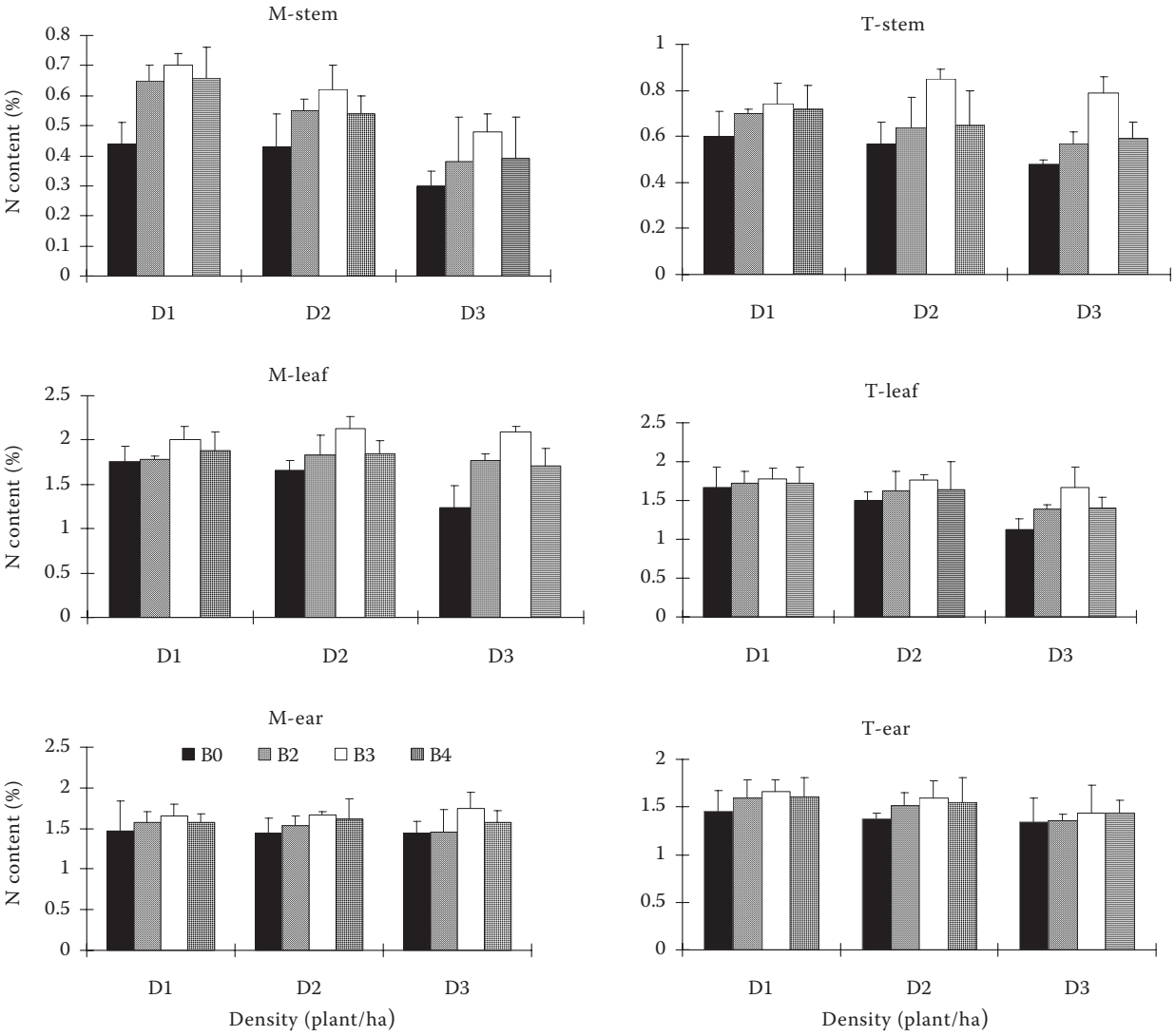

Figure 1. Effect of uniconazole and densities on N content in tissues of main stem (M, left) and tillers (T, right) at mature stage. D1, D2, and D3 represent densities levels of 90×10^4, 180×10^4, and 270×10^4 per ha, respectively; B0, B2, B3, and B4 represent 0, 10, 20, and 40 mg/kg uniconazole was applied to seed, respectively

Grain protein content and protein composition. Uniconazole concentration had a relatively significant effect on grain protein content (Table 3). Mean grain protein content increased with increasing uniconazole concentrations; the total protein content in the case of B0, B1, B2, and B3 were 13.96, 14.11, 14.26, and 14.38%, respectively. However, density had a reverse influence on grain protein; grain protein reduced as density increased. Grain protein content at three density levels at uniconazole concentrations B1, B2, and B3 were partly different compared with control B0; D3 resulted in the highest increase. Therefore, it was obvious that the best uniconazole disposals for protein content were at high density (D3).

Significant (LSD, $P < 0.05$) variation in concentrations of gliadins, glutenins, albumins, and globulins was also related to uniconazole concentrations and plant density. Variation was the most evident when the influence of uniconazole on protein content was significant. There was a linear increase in the relative proportion of gliadin and glutenin proteins as flour protein increased (Table 4). As flour uniconazole concentrations increased, the proportion of gliadins increased more rapidly than that of glutenins. SS proof-test result indicated, the effects of uniconazole on gliadins and glutenins were bigger than that of densities.

As Table 4 indicates, albumins and globulins represented an unsimilar trend along with the density increased. Uniconazole concentrations significantly (LSD, $P < 0.05$) increased the content of albumins, globulins, and its summation at different densities; among them, the highest rise range was at B2 treatment. The rate of changes in albumins and globulins elevated with

Table 3. Effect of uniconazole and plant density on total protein content (%)

Treatment	B0	B2	B3	B4	Average
D1	14.63	14.71	14.79	14.87	14.75[a]
D2	13.82	14.07	14.15	14.31	14.09[b]
D3	13.44	13.56	13.80	13.96	13.69[c]
Average	13.96[d]	14.11[c]	14.26[b]	14.38[a]	

Each result is the average of three repetitions. Values followed by the same letter in the same column are not significantly different. Different small letters indicate significance at the 0.05 level. D1, D2, D3 – density levels of 90 × 10^4, 180 × 10^4, and 270 × 10^4 per ha, respectively; B0, B2, B3, B4 – 0, 10, 20, and 40 mg/kg uniconazole applied to seed, respectively

the concentrations increased at low density; at higher density, the effects of B3 were greater than that of B4.

SDS sedimentation volumes and WGC. SDS sedimentation volumes were highly correlated with grain protein. When uniconazole concentration response was significant (LSD, $P < 0.05$), an increase in SDS volume was evident. Uniconazole effects on SDS at different densities were D1 > D3 > D2. In contrast, density reduced sedimentation volume (Table 5).

WGC was significantly (LSD, $P < 0.05$) influenced by uniconazole concentration. Increasing uniconazole concentration contributed to significantly (LSD, $P < 0.05$) increased WGC content at three densities. WGC was also significantly (LSD, $P < 0.05$) influenced by main effects of density (Table 5). However, this may simply be a function of limited factor. Uniconazole concentration for high density seems to be the best strategy.

Flour dough farinographic properties. The results of the farinographic studies are shown in Table 6. WA, which represents the amount of water required to center the farinogram curve on the 500 BU line, increased steadily with each increment of flour in the blends. D1 flour showed larger WA values as compared to other density flours. The uniconazole concentration flours generated an average increment same as WGC. Table 6 also presents the results of dough development time (DDT) and dough stability time (DST). Wheat flour treatment with uniconazole resulted in larger values for all density levels compared to the control samples. Density also changed DDT and DST, but in smaller extent than uniconazole. Applied uniconazole could prolong DBT, whereas increased density could shorten it. DBT ought to exist at 10–14 min. Treatments that reached the range were D1B0, D2B0, D2B2, D3B1; in other treatments DBT occurred either before the time or overtime (Table 6). It indicates that the influence of uniconazole on DBT was disadvantaged at lower density level, but low concentrations were appropriate to higher density level. Table 6 also shows that uniconazole contributed to longer DBT, although MTI and DS were relatively decreased.

Table 4. Effect of uniconazole and plant density on wheat protein compositions

Treatment	Gliadin (%)				Glutenin (%)				Albumin (%)				Globulin (%)			
	D1	D2	D3	$\bar{x}$	D1	D2	D3	$\bar{x}$	D1	D2	D3	$\bar{x}$	D1	D2	D3	$\bar{x}$
B0	4.32	4.18	4.11	4.20[d]	5.23	5.05	4.90	5.06[c]	1.11	1.06	1.08	1.08[c]	0.60	0.50	0.45	0.52[c]
B2	4.67	4.47	4.50	4.55[c]	5.40	5.38	5.37	5.38[b]	1.13	1.14	1.13	1.13[b]	0.59	0.52	0.49	0.53[b]
B3	4.65	4.59	4.52	4.59[b]	5.43	5.40	5.43	5.42[b]	1.29	1.17	1.15	1.20[a]	0.64	0.54	0.55	0.58[a]
B4	4.72	4.68	4.58	4.66[a]	5.97	5.89	5.85	5.90[a]	1.38	1.05	1.02	1.15[b]	0.73	0.50	0.48	0.57[a]
Average	4.60[a]	4.48[b]	4.42[c]		5.51[a]	5.44[b]	5.39[b]		1.23[a]	1.11[b]	1.10[b]		0.64[a]	0.52[b]	0.49[c]	

Each result is the average of three repetitions. Values followed by the same letter in the same column are not significantly different. Different small letters indicate significance at the 0.05 level. D1, D2, D3 – density levels of 90 × 10^4, 180 × 10^4, and 270 × 10^4 per ha, respectively; B0, B2, B3, B4 – 0, 10, 20, and 40 mg/kg uniconazole applied to seed, respectively

Table 5. Effect of uniconazole and plant density on SDS and WGC

Treatment	SDS (ml)				WGC(%)			
	D1	D2	D3	average	D1	D2	D3	average
B0	39.0	38.0	34.0	37.00[d]	31.94	31.80	31.41	31.72[d]
B2	42.3	38.0	36.5	38.93[c]	32.20	32.53	31.92	32.22[c]
B3	43.9	39.0	37.0	39.97[b]	33.60	33.70	32.96	33.42[b]
B4	48.4	43.5	40.6	44.17[a]	36.34	34.88	33.15	34.79[a]
Average	43.4[a]	39.6[b]	37.0[c]	–	33.52[a]	33.23[b]	32.36[c]	–

SDS – sedimentation volumes, WGC – wet gluten content. Each result is the average of three repetitions. Values followed by the same letter in the same column are not significantly different. Different small letters indicate significant at the 0. 05 level. D1, D2, and D3 – densities levels of 90×10^4, 180×10^4 , and 270×10^4/ ha, respectively; B0, B2, B3, and B4 – 0, 10, 20, and 40 mg/kg uniconazole was applied to seed, respectively.

Table 6. Effect of uniconazole and plant density on flour dough farinographic properties

Treatment		WA (%)	DDT (min)	DST (min)	MTI	DBT (min)	DS (BU)	VV
D1	B0	62.8	6.0	8.7	30	14.1	48	64
	B2	63.6*	8.3*	7.2	20*	21.5**	22**	74*
	B3	62.8	7.3*	6.8*	33	17.0*	45	68
	B4	62.8	11.5**	15.0**	10**	21.5**	45	81**
D2	B0	63.2	5.1	6.1	40	10.0	66	59
	B2	62.0*	6.2*	4.7*	26**	14.4**	56*	62
	B3	62.4	7.4**	7.0*	40	11.5	60	68*
	B4	62.8	8.1**	14.5**	16**	19.3**	56	72**
D3	B0	63.0	4.4	4.5	50	6.9	82	54
	B2	62.6	5.5*	5.2*	35*	12.5**	55**	61*
	B3	62.0	5.5*	6.1*	26**	15.0**	46**	62*
	B4	64.0**	7.3**	15.7**	10**	23.0**	20**	72**

WA – water absorption, DDT – dough development time, DST – dough stability time, MTI – mixing tolerance index, DBT – dough breakdown time, DS – degree of softening, VV – valorimeter value. Each result is the average of three repetitions. *$P < 0.05$, **$P < 0.01$; D1, D2, D3 – density levels of 90×10^4, 180×10^4, and 270×10^4 per ha, respectively; B0, B2, B3, B4 – 0, 10, 20, and 40 mg/kg uniconazole applied to seed, respectively

Valorimeter value (VV) was the colligate reflection of wheat flour character. The higher the VV, the better was the flour quality. The results of this experiment showed that uniconazole increased VV, but density reduced VV. The effect of uniconazole on VV was D3 > D1 > D2; from high density to low density, the average advanced scope partly were 20.3%, 14.1%, 16.1% than that of the control. According to VV, wheat dough was divided into Strong Gluten flour (over 65), Middle gluten flour (60–65), and Weak gluten flour (less than 50). As seen from Table 5, at this experiment the treatments which reached Strong Gluten flour were: D1B1, D1B2, D1B3, D2B2, D2B3, and D3B3. Thereby, uniconazole contributed to an increase of VV at relatively low density; at relatively high density higher concentrations were advantage to increase VV.

DISCUSSION

One of the main results of this experiment may be the yield and grain protein of winter wheat

in South China which may be regulated through nitrogen source regulation with uniconazole. The unusual environments of Sichuan Basin are a challenge for maintaining consistent high grain yields and high grain protein. Seed development drew on dry matter and particularly N mobilized from the stem and leaf (Hocking et al. 1997), and the combined mobilization from stem + leaf could have contributed 56.4% of the N content of seeds. The study of Barlow et al. (1983) indicated that the sediment velocity of protein rest on the level of substances supply. This experiment showed that uniconazole had effects on harmonizing N distribution in stem, leaf and ear. The contents of N accumulated in ear, stem and leaf were higher in uniconazole concentrations than in control, and the effect of uniconazole on main stem was bigger than that on tillers. The results of this experiment show that stem contained only about 50% of the plant N compared to leaves, reflecting the lower N concentration in the stem, which was similar to the results of Hocking et al. (1997). Yet, it differs from the results of Smith et al. (1988), who showed that stems at flowering contained a greater proportion of the plant N than the leaves; however, dead leaves were not included in their data. Therefore, their values for leaf N as a proportion of total plant N would be underestimated.

Beside the aim of achieving high grain yields, grain quality plays an important role for crop farmers since quality requirements influence prices received by farmers. Grain protein concentration and composition have long been recognized as major traits determining cereals end-use value. Several crop simulation models simulate the accumulation of grain dry mass and total N, and thus protein concentration (Porter 1993, Brisson et al. 1998, Jamieson and Semenov 2000, Asseng et al. 2002). However, South China wheat was associated with weaker dough properties compared with North China wheat. Flour gluten was a factor deteriorating flour quality in Asian wheat cultivars. Improvement of flour gluten in the wheat breeding programs in China would be significant in developing Chinese wheat cultivars with better quality. As to flour gluten, seen from VV, the results of this study showed that at the presence of uniconazole there was an increase in the activities of VV. Currently, the reason for such increase of VV affected by uniconazole is unknown. The grain protein was significantly higher in uniconazole concentrations than in control. With the increase of uniconazole concentrations, the contents of grain protein gliadin and glutenin increased significantly, and the contents of albumin and globulin increased as well. Uniconazole at 20 mg/kg was the most favorable for improving grain protein and protein fractions. These results strongly suggest that genotype-by-environment interactions for grain protein composition act mostly via variations of total N per grain, i.e. N availability, and not via the regulation of protein synthesis in the grain.

Significant interactions of density with uniconazole were observed for yield, protein quality, protein composition, and dough mixing properties. However, protein quality and composition responded little to low density and low uniconazole treatments. This reinforces the importance of crop management strategies to reach desired marketing targets for flour quality and end-product performance.

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