

# Effects of nitrogen application stage on grain yield and nitrogen use efficiency of high-yield summer maize

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## ABSTRACT

This study aims to explore the optimum nitrogen (N) application method by analyzing effects of variable N application stages and ratios on the N absorption and translocation of high-yield summer maize (DH661). The study included field experiments and  $^{15}\text{N}$  isotopic dilutions for pot experiments. Results showed that the yield was not increased in a one-off N application at the jointing stage. The uptake of fertilizer-derived N in the grain increased with the increasing of N applied times. Compared to a single or double application, total N uptake ( $N_{\text{up}}$ ) and biomass increased significantly by supplying N at the six-leaf stage (V6), ten-leaf stage (V10) and 10 days after anthesis in ratios of 3:5:2 and 2:4:4. The fertilizer-derived recovery rates were 67.5% and 78.1%, respectively. The uptake and utilization of fertilizer-derived N was enhanced by increasing the recovery rate of N supplied after anthesis, and reducing the absorption of soil-derived N. Therefore, the 2:4:4 application ratios was the optimal N application method.

**Keywords:** N application method;  $^{15}\text{N}$  isotope tracer; N uptake and translocation; soil N; N fertilizer

During the past half-century, food production has increased obviously, owing to nitrogen (N) fertilizers (Dobermann and Cassman 2005). However, crops production would not increase unlimitedly as rising applied N, worldwide nitrogen use efficiency (NUE) is approximately 33% (Raun and Johnson 1999). More attention was paid to the increasing N application rationally (Galloway et al. 2004). Since the 1980s, N recommendation for maize production in China relied extensively on a yield-based strategy, developed to represent regional averages. In recent years, issues for maize production such as excessive N application and ineffective application stage strategies have become common in the pursuit of high yield. As a result, overall efficiency has decreased. In 2004 the domestic N recovery efficiency of maize farmland was just 26.1% (Ma et al. 2009). Nutrient use efficiencies of the major cereal crops was obviously much lower than the world average, which is due to the excessive use of chemical fertilizer and the ignorance of nutrients contribution from the cultivated techniques and the soil (Zhang et al. 2008).

The average yield of summer maize in the Shandong Province was 6586 kg/ha in 2009. Since 2007 the yield of small area land has exceeded 15 t/ha in more areas, but the N rate was more than 457.9 kg/ha (Zhang et al. 2008). However, high N rate in exchange for high yield causes wasted resources and environmental pollution. In addition, the production process typically uses a method of one-off N applied at V6, leading to wilting at the seedling stage and decreasing the yield or the NUE. So it is necessary to manage N application for summer maize scientifically (Hawkins et al. 2007). The yield may be increased by using split application method under irrigated conditions (Randall et al. 2003, Gehl et al. 2005). Cui et al. (2008) reported that improved soil N management based on the minimum N requirement can be applied to summer maize production in the North China Plain.

The objective of this paper was to study the effects of variable N application stages and ratios on the uptake and translocation of N in high-yield

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Table 1. The test soil nutrients content before sowing and the climatic conditions during the growing period

	Soil type	Total N (g/kg)	Alkaline hydrolysable N (mg/kg)	Rapidly available P (mg/kg)	Exchangeable K (mg/kg)	Organic matter (g/kg)	Rainfall (mm)	Average temperature (°C)	Sunlight (h)
Field	08 brown soil	0.94	86.9	57.4	113.45	9.21	459.1	23.8	813.0
	09 brown soil	0.95	86.5	62.4	109.36	9.21	463.8	23.9	809.2
Pot	09 brown soil	0.61	64.5	47.8	102.19	0.85	463.8	23.9	809.2

summer maize, and to explore the optimal N application method.

## MATERIAL AND METHODS

**Plant material and experimental site.** The experiment was done in the test farm of the Shandong Agricultural University (36°09'N, 117°09'E). The field experiment was done in 2008 and 2009. The pot experiment was done in 2009, and the dimensions of a pot are 37 cm (diameter) × 50 cm (height). Each pot was composed topsoil (0–20 cm deep), blended, cribrated and mixed with river sand in a 5:1 volume ratio. The soil nutrients and climatic conditions were listed in Table 1. Denghai661 (DH661) was used as experimental material. The previous crop was winter wheat, sown on June 12<sup>th</sup> and harvested on October 5<sup>th</sup>.

### Experimental design

**Field experiment.** Planting density was 75 000 plants/ha, with equal row spacing of 60 cm. The

each plot area was 100 m<sup>2</sup>, including 3 replicate test areas arranged randomly. The amount of applied N was 360 kg/ha. Urea, 46% N content, was used as the N fertilizer. 857 kg/ha of triple superphosphate and 400 kg/ha of KCl were applied at the pre-sowing and V6, respectively.

**Pot experiment.** The pots were filled 30 days before sowing, sunk steadily and then placed in square pits which were 45 cm deep. Distance between plants was 37 cm, with 60 cm row spacing. Guard rows were planted around the pits. The amount of applied N was 4.8 g/plant (which is equivalent to 360 kg N/ha in the field experiment planted by 75 000 plants/ha), and the N fertilizer used was <sup>15</sup>N urea (the <sup>15</sup>N content is 10.41%). In addition, 11.4 g/plant of triple superphosphate and 5.3 g/plant KCl were applied at the pre-sowing and V6, respectively. The N application stages and ratios are shown in Table 2.

**Analytical measurements.** Three plant samples were taken continuously from each treatment at the V6, V10, tasseling stage (VT) and maturity stage (R6). The root was preserved after rinsing clean. The whole plants were preserved at V6, while plants were separated into leaf and stem sheath

Table 2. Nitrogen application stages and ratios of different treatments

Treatments	V6		V10		10 days after anthesis		
	N application ratio (%)	urea type	N application ratio (%)	urea type	N application ratio (%)	urea type	
T1	I	0	0		0		
T2	I	100	15N	0	0		
T3	I	40	15N	60	general	0	
	II	40	general	60	15N	0	
T4	I	30	15N	50	general	20	general
	II	30	general	50	15N	20	general
	III	30	general	50	general	20	15N
T5	I	20	15N	40	general	40	general
	II	20	general	40	15N	40	general
	III	20	general	40	general	40	15N

The urea type of the field experiment was the general urea in all. V6 – six-leaf stage; V10 – ten-leaf stage

at V10 and VT, and were divided into leaf, stem sheath, ear bract, the cob, and the grain at R6. After dried at 85°C, plant samples were pulverized and determined after weighing. The yield was estimated at maturity. Total N was measured by the continuous flow analytical system of AutoAnalyzer 3-AA3 (Isoprime, Manchester, UK) after sulfuric acid-hydrogen peroxide digestion of plant sample. <sup>15</sup>N content was measured by an IsoPrime100 stable isotope analyzer (Camin et al. 2007). The determination procedures as follows: weighing 5.00 mg of pulverized plant samples, packed up by the tinfoil paper and placed in the automatic sample feeding disc, burned fully into the gas in combustion tube, the gas was imported to the measuring tank and determined by standard sample of <sup>15</sup>N.

**Evaluation of nitrogen uptake efficiency.** The following parameters were derived according to Moll et al. (1982):

N Harvest Index (NHI, %) =  $N_{up}$  by grain/total  $N_{up}$  by plant × 100;

N Agriculture Efficiency (NAE, g grain/g  $N_p$ ) = (grain yield with added N – grain yield with no N)/N application amount;

N recovery efficiency (NRE, %) = (plant N absorption amount with added N – plant N absorption amount with no N)/N application amount × 100;

N partial factor productivity (NPF, g grain/g  $N_p$ ) = plant yield with added N application/N application amount;

N proportion derived from fertilizer (NDF, %) =  $^{15}N$  atom percentage excess in plant (%) /  $^{15}N$  atom percentage excess in fertilizer (%) × 100;

N amount derived from fertilizer (NDF, mg) = dry weight of organ (g) × total N in organ (%) × NDF (%) × 1000/104;

N distribution rate (NDR, %) = NDF of organ (mg)/NDF of plant (mg) × 100;

N amount derived from soil (NDFS, mg) = total N of plant (mg) – NDF (mg);

N proportion derived from soil (NDFS, %) = 100% – NDF (%) × 100;

N recovery rate (NRR, %) = NDF of plant (mg)/N application amount (mg) × 100;

N translocation efficiency (NTE, %) = N translocation in vegetative organs/ $N_{up}$  by vegetative organs at anthesis × 100;

N assimilation amount after anthesis (NAAA, kg/ha) =  $N_{up}$  by grains at maturity stage – N translocation in vegetative organs.

**Statistical analysis.** All data were analyzed using LSD tests, within the SPSS 16.0 Statistical Software Package. Results were considered significant in all statistical calculations where  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

**Yield, NHI, NAE and NPF.** Results of the two-year field experiment are consistent (Table 3). Yield of one-off applied N at V6 (T2) was not increased significantly compared to T1. The yield improved with an increasing N application ratio (T3 lowest, T5 highest). In the field experiment, the yield of T5 increased by 15.3%, 14.2% and 7.1% compared to T1, T2 and T3, respectively. In the pot experiment, the yield increased compared to T1, T2 and T3 by 58.2%, 56.9% and 27.6%. Additionally, the NHI, the NAE and NPF were influenced, and marked by T5 > T4 > T3 > T2 remarkably. So splitting applications of N could significantly increase the yield, the NHI, the NAE and the NPF of DH661.

In 2006 and 2007, the average yield of the top three in Corn High Yield Contest in America were 17 297.2 kg/ha and 18 749.5 kg/ha. The average applied N rate was 284.5 kg/ha and 300 kg/ha, 51.85% of which was applied before sowing and supplied several times during the growing period. So the preferable method was to apply N in 3:5:2 and 2:4:4 ratios at the V6, V10 and grains filling stage, respectively.

**N uptake and distribution from different sources.** The whole plant  $N_{up}$  of T2 and T3 at V10 and VT were higher than the other treatments. At VT,  $N_{up}$  decreased with the increasing applied N ratio (T2 > T3 > T4 > T5). After VT,  $N_{up}$  of T4 and T5 were significantly increased. Compared to T1 and T2, total N of T5 was increased by 44.6% and 11.9%. Compared to T2, T3 and T4, NDF of T5 was enhanced by 55.1%, 22.8%, and 15.7%, respectively. Accordingly, the proportion of NDF in the total  $N_{up}$  of T5 was also remarkably higher, but NDFS decreased (T2 > T3 > T4 > T5), as shown in Table 4.

The  $N_{up}$  of the grains, leaf blade, stem sheath, root system, ear axis and the bud leaf at maturity decreased. With an increasing N application ratio, NDF reduced significantly in the leaf blade and in the stem sheath, but increased in the grain. NDR of the grain in T5 was higher than in other treatments, compared to T2, T3, and T4, NDF of T5 increased by 165.5%, 74.2% and 35.8%, the grain NDR of T5 was increased by 31.2%, 22.2% and 11.1%, respectively (Table 5).

The N uptake and distribution after flowering was important for the grain filling of crops. The  $N_{up}$  ratio before and after anthesis was 48:52 in high yield summer maize, whereas it was 76:24 in conventional summer maize. It was essential to improve the yield to maintain higher grain-filling

Table 4. Effects of N application stage on  $N_{up}$  from different source

Growth stages	Treatments	Total $N_{up}$ (mg/plant)	$N_s$ at V6 (mg/plant)	$N_s$ at V10 (mg/plant)	$N_s$ at 10 days after anthesis (mg/plant)	NDFE		NDFS	
						amount (mg/plant)	proportion (%)	amount (mg/plant)	proportion (%)
V6	T1	220.5 <sup>a</sup>						220.5 <sup>a</sup>	100 <sup>a</sup>
	T2	219.1 <sup>a</sup>						219.1 <sup>a</sup>	100 <sup>a</sup>
	T3	221.6 <sup>a</sup>						221.6 <sup>a</sup>	100 <sup>a</sup>
	T4	220.3 <sup>a</sup>						220.3 <sup>a</sup>	100 <sup>a</sup>
	T5	219.7 <sup>a</sup>						219.7 <sup>a</sup>	100 <sup>a</sup>
V10	T1	409.7 <sup>d</sup>						409.7 <sup>c</sup>	100 <sup>a</sup>
	T2	1157.9 <sup>a</sup>	441.2 <sup>b</sup>			441.2 <sup>b</sup>	38.1 <sup>c</sup>	716.7 <sup>a</sup>	61.9 <sup>a</sup>
	T3	1010.7 <sup>b</sup>	621.6 <sup>a</sup>			621.6 <sup>a</sup>	61.5 <sup>a</sup>	389.1 <sup>d</sup>	38.5 <sup>c</sup>
	T4	1106.1 <sup>a</sup>	634.0 <sup>a</sup>			633.9 <sup>a</sup>	57.3 <sup>a</sup>	472.2 <sup>b</sup>	42.7 <sup>c</sup>
	T5	884.3 <sup>c</sup>	423.2 <sup>b</sup>			423.2 <sup>b</sup>	47.9 <sup>b</sup>	461.1 <sup>b</sup>	52.1 <sup>b</sup>
VT	T1	1491.8 <sup>e</sup>						1491.8	100 <sup>a</sup>
	T2	2842.0 <sup>a</sup>	1147.7 <sup>a</sup>			1147.7 <sup>b</sup>	40.4 <sup>c</sup>	1694.2 <sup>a</sup>	59.6 <sup>b</sup>
	T3	2649.1 <sup>b</sup>	652.0 <sup>c</sup>	413.8 <sup>c</sup>		1065.8 <sup>b</sup>	40.2 <sup>c</sup>	1583.3 <sup>a</sup>	59.8 <sup>b</sup>
	T4	2332.7 <sup>c</sup>	746.1 <sup>b</sup>	818.1 <sup>a</sup>		1564.2 <sup>a</sup>	67.1 <sup>a</sup>	768.5 <sup>c</sup>	32.9 <sup>d</sup>
	T5	2100.1 <sup>d</sup>	525.0 <sup>d</sup>	615.6 <sup>b</sup>		1140.6 <sup>b</sup>	54.3 <sup>b</sup>	959.5 <sup>b</sup>	45.7 <sup>c</sup>
R2	T1	1997.2 <sup>c</sup>						1997.3 <sup>a</sup>	100 <sup>a</sup>
	T2	3303.0 <sup>b</sup>	1985.4 <sup>a</sup>			1985.4 <sup>c</sup>	60.1 <sup>c</sup>	1317.6 <sup>b</sup>	39.9 <sup>b</sup>
	T3	3475.6 <sup>b</sup>	989.4 <sup>b</sup>	1349.7 <sup>a</sup>		2339.1 <sup>b</sup>	67.3 <sup>b</sup>	1136.5 <sup>d</sup>	32.7 <sup>c</sup>
	T4	3642.8 <sup>ab</sup>	892.6 <sup>d</sup>	1139.4 <sup>b</sup>	770.1 <sup>b</sup>	2802 <sup>a</sup>	76.9 <sup>a</sup>	840.8 <sup>e</sup>	23.1 <sup>d</sup>
	T5	3672.5 <sup>a</sup>	657.8 <sup>c</sup>	787.9 <sup>c</sup>	1023.5 <sup>a</sup>	2469.2 <sup>b</sup>	67.2 <sup>b</sup>	1203.3 <sup>c</sup>	32.8 <sup>c</sup>
R6	T1	4422.3 <sup>c</sup>						4422.3 <sup>a</sup>	100 <sup>a</sup>
	T2	5715.0 <sup>b</sup>	2417.2 <sup>a</sup>			2417.2 <sup>d</sup>	42.3 <sup>b</sup>	3297.8 <sup>b</sup>	57.7 <sup>b</sup>
	T3	6234.6 <sup>a</sup>	1355.2 <sup>b</sup>	1697.5 <sup>a</sup>		3052.8 <sup>c</sup>	49.0 <sup>b</sup>	3181.8 <sup>b</sup>	51.0 <sup>bc</sup>
	T4	6350.2 <sup>a</sup>	976.0 <sup>c</sup>	1398.0 <sup>b</sup>	866.8 <sup>b</sup>	3240.7 <sup>b</sup>	51.0 <sup>b</sup>	3109.5 <sup>b</sup>	49.0 <sup>c</sup>
	T5	6396.4 <sup>a</sup>	668.8 <sup>d</sup>	1477.9 <sup>b</sup>	1601.5 <sup>a</sup>	3748.0 <sup>a</sup>	58.6 <sup>a</sup>	2648.4 <sup>c</sup>	41.4 <sup>d</sup>

Ns – N supplied. The data were from the pot experiment in 2009 if the experiment type is not noted. NDFE – N proportion derived from fertilizer; NDFS – N amount derived from soil

rate and longer duration of active growing period. The results showed that splitting applications of N could increase the total  $N_{up}$  of the plant by enhancing the NDFE, and increasing the grain NDR.

**N recovery efficiency and N recovery rate.** The NRE of T5 in the field experiment was improved by 23.6% and 18.0% compared to T2 and T3, respectively. For the pot experiment, compared to T2, the NRE of T3, T4, and T5 was increased by 10.8%, 13.2% and 14.2%. Applying N at different stages had significant impacts on the recovery rate of NDFE. The NRR of T5 was higher than other treatments, increasing by 27.7%, 14.5% and 10.6% compared to T2, T3 and T4.

The  $N_{up}$  after V10 in high-yield varieties accounted for over 60% of the total uptake (Subedi and Ma 2005). N deficiency reduced the yield and  $N_{up}$  in later stages of development (Rajcan and Tollenaar 1999). The N requirement of grain was the main force for uptake at the grain filling stage. The average NRR at the V6 and V10 were 64.6% and 64.7%, and 86.9% after anthesis. Therefore, the higher NRR after anthesis led to higher overall NRR in T4 and T5 (Table 6).

**Nitrogen translocation efficiency.** The grain filling stage is a crucial stage not only for grain formation but also for the N absorption and transport in the plant. Osaki et al. (1991) reported that

Table 5.  $N_{up}$  efficiency from different sources and N distribution rate (NDR) at maturity

Organs	Treatments	$N_s$	$N_s$	$N_s$ at 10 days	NDFD		NDFS		NDR (%)
		at V6	at V10	after anthesis	DA (mg/p)	DP (%)	DA (mg/p)	DP (%)	
Leaf	T1						778.6 <sup>b</sup>	100 <sup>a</sup>	
	T2	489.6 <sup>a</sup>			489.6 <sup>b</sup>	36.2 <sup>d</sup>	863.7 <sup>a</sup>	63.8 <sup>b</sup>	20.3 <sup>a</sup>
	T3	325.1 <sup>b</sup>	202.2 <sup>a</sup>		527.3 <sup>a</sup>	58.2 <sup>a</sup>	378.9 <sup>d</sup>	41.8 <sup>e</sup>	17.3 <sup>b</sup>
	T4	179.8 <sup>c</sup>	163.3 <sup>b</sup>	51.6 <sup>b</sup>	394.7 <sup>c</sup>	51.4 <sup>b</sup>	372.5 <sup>d</sup>	48.6 <sup>d</sup>	12.2 <sup>c</sup>
	T5	148.6 <sup>c</sup>	104.4 <sup>c</sup>	70.5 <sup>a</sup>	323.5 <sup>c</sup>	43.4 <sup>c</sup>	421.2 <sup>c</sup>	56.6 <sup>c</sup>	8.6 <sup>d</sup>
Stem and sheath	T1						426.3 <sup>c</sup>	100 <sup>a</sup>	
	T2	472.5 <sup>a</sup>			472.5 <sup>a</sup>	51.3 <sup>a</sup>	449.2 <sup>b</sup>	48.7 <sup>d</sup>	19.6 <sup>a</sup>
	T3	220.0 <sup>b</sup>	157.0 <sup>a</sup>		377.0 <sup>b</sup>	46.0 <sup>b</sup>	441.8 <sup>b</sup>	54.0 <sup>c</sup>	12.4 <sup>b</sup>
	T4	149.3 <sup>c</sup>	143.9 <sup>a</sup>	33.1 <sup>b</sup>	326.3 <sup>c</sup>	37.6 <sup>c</sup>	542.6 <sup>a</sup>	62.4 <sup>b</sup>	10.1 <sup>b</sup>
	T5	52.7 <sup>d</sup>	108.1 <sup>b</sup>	108.2 <sup>a</sup>	269.0 <sup>d</sup>	38.9 <sup>c</sup>	423.3 <sup>c</sup>	61.1 <sup>b</sup>	7.2 <sup>c</sup>
Bract	T1						113.8 <sup>a</sup>	100 <sup>a</sup>	
	T2	57.1 <sup>a</sup>			57.1 <sup>a</sup>	50.2 <sup>b</sup>	67.2 <sup>b</sup>	49.8 <sup>c</sup>	2.4 <sup>a</sup>
	T3	20.5 <sup>b</sup>	20.0 <sup>b</sup>		40.5 <sup>b</sup>	32.6 <sup>c</sup>	42.3 <sup>c</sup>	67.4 <sup>b</sup>	1.3 <sup>b</sup>
	T4	19.8 <sup>b</sup>	22.1 <sup>a</sup>	7.1 <sup>b</sup>	59.0 <sup>a</sup>	71.3 <sup>a</sup>	36.7 <sup>d</sup>	28.7 <sup>d</sup>	1.5 <sup>b</sup>
	T5	9.3 <sup>c</sup>	14.1 <sup>c</sup>	12.8 <sup>a</sup>	36.2 <sup>c</sup>	37.8 <sup>c</sup>	39.5 <sup>d</sup>	62.2 <sup>b</sup>	1.0 <sup>c</sup>
Ear axis	T1						302.7 <sup>a</sup>	100 <sup>a</sup>	
	T2	115.3 <sup>a</sup>			115.3 <sup>b</sup>	38.1 <sup>b</sup>	177.7 <sup>c</sup>	61.9 <sup>c</sup>	4.8 <sup>b</sup>
	T3	69.3 <sup>b</sup>	88.7 <sup>a</sup>		158.0 <sup>a</sup>	53.9 <sup>a</sup>	170.2 <sup>c</sup>	46.1 <sup>d</sup>	5.2 <sup>a</sup>
	T4	68.1 <sup>b</sup>	79.7 <sup>b</sup>	22.0 <sup>b</sup>	169.8 <sup>a</sup>	51.7 <sup>a</sup>	173.8 <sup>c</sup>	48.3 <sup>d</sup>	5.2 <sup>a</sup>
	T5	27.1 <sup>c</sup>	37.7 <sup>c</sup>	32.7 <sup>a</sup>	97.5 <sup>c</sup>	28.4 <sup>c</sup>	206.3 <sup>b</sup>	71.6 <sup>b</sup>	2.6 <sup>c</sup>
Grain	T1						2256.9 <sup>a</sup>	100.0 <sup>a</sup>	
	T2	1059.5 <sup>a</sup>			1059.5 <sup>d</sup>	46.9 <sup>c</sup>	1449.0 <sup>c</sup>	53.1 <sup>b</sup>	43.8 <sup>d</sup>
	T3	579.1 <sup>b</sup>	1035.7 <sup>a</sup>		1614.8 <sup>c</sup>	64.4 <sup>b</sup>	1820.9 <sup>b</sup>	35.6 <sup>b</sup>	52.9 <sup>c</sup>
	T4	454.1 <sup>c</sup>	885.7 <sup>b</sup>	732.7 <sup>b</sup>	2072.5 <sup>b</sup>	60.3 <sup>b</sup>	1439.2 <sup>c</sup>	39.7 <sup>b</sup>	64.0 <sup>b</sup>
	T5	354.7 <sup>d</sup>	1119.8 <sup>a</sup>	1339.0 <sup>a</sup>	2813.5 <sup>a</sup>	80.1 <sup>a</sup>	1128.9 <sup>d</sup>	19.9 <sup>c</sup>	75.1 <sup>a</sup>
Root	T1						544.0 <sup>a</sup>	100 <sup>a</sup>	
	T2	223.2 <sup>a</sup>			223.2 <sup>b</sup>	41.0 <sup>b</sup>	291.1 <sup>d</sup>	59.0 <sup>c</sup>	9.2 <sup>b</sup>
	T3	141.2 <sup>b</sup>	193.9 <sup>a</sup>		335.1 <sup>a</sup>	65.2 <sup>a</sup>	327.9 <sup>c</sup>	34.8 <sup>d</sup>	11.0 <sup>a</sup>
	T4	104.9 <sup>c</sup>	97.3 <sup>b</sup>	26.3 <sup>b</sup>	228.5 <sup>b</sup>	34.5 <sup>c</sup>	414.7 <sup>b</sup>	65.5 <sup>b</sup>	7.1 <sup>c</sup>
	T5	76.4 <sup>d</sup>	93.8 <sup>b</sup>	38.3 <sup>a</sup>	208.5 <sup>c</sup>	32.4 <sup>c</sup>	429.0 <sup>b</sup>	67.6 <sup>b</sup>	5.6 <sup>d</sup>

DA – distribution amount; DP – distribution proportion;  $N_s$  – N supplied; NDFD – N proportion derived from fertilizer; NDFS – N amount derived from soil

grain N was partly derived from the N accumulated in the stem and leaves before male tetrad stage and partly redirected from the root system after flowering. With an increasing N application ratio in the field experiment, NAAA of grain increased at maturity. Total  $N_{up}$  in the grain of T5 was increased by 62.8%, 52.1% and 36.8%, compared to

T1, T2 and T3, respectively. NTE of T3 was higher than that of T2, but the NAAA was significantly reduced, which was unfavorable to the overall uptake in the grain. Supplying N after anthesis enhanced the  $N_{up}$  in grain significantly. NAAA of T5 was the highest, enhanced by 118.3%, 73.1% and 63.8% compared to T1, T2 and T3 (Table 6).

Table 6. Effects of N application stage on N recovery efficiency (NRE), N recovery rate (NRR), N assimilation amount after N assimilation amount after anthesis (NAAA) and N translocation efficiency (NTE)

Treat- ments	NRE (%)			NRR (%)			NDFE	N <sub>up</sub> by grain (kg/ha)		NAAA (kg/ha)		NTE (%)	
	field		pot 09	N <sub>s</sub> at V6	N <sub>s</sub> at V10	N <sub>s</sub> at 10 days after anthesis		08	09	08	09	08	09
	08	09											
T1							166.7 <sup>e</sup>	176.1 <sup>c</sup>	82.8 <sup>e</sup>	80.4 <sup>e</sup>	45.1 <sup>b</sup>	51.5 <sup>b</sup>	
T2	8.99 <sup>e</sup>	9.56 <sup>e</sup>	26.9 <sup>b</sup>	50.4 <sup>b</sup>			50.4 <sup>d</sup>	178.4 <sup>d</sup>	188.5 <sup>c</sup>	103.2 <sup>d</sup>	102.6 <sup>d</sup>	38.0 <sup>c</sup>	43.4 <sup>c</sup>
T3	14.13 <sup>d</sup>	15.61 <sup>c</sup>	37.8 <sup>a</sup>	70.6 <sup>a</sup>	58.9 <sup>b</sup>		63.6 <sup>c</sup>	204.4 <sup>c</sup>	203.5 <sup>c</sup>	106.8 <sup>cd</sup>	110.7 <sup>c</sup>	45.9 <sup>b</sup>	43.6 <sup>c</sup>
T4	22.86 <sup>c</sup>	24.31 <sup>d</sup>	40.2 <sup>a</sup>	67.8 <sup>a</sup>	58.3 <sup>b</sup>	90.3 <sup>a</sup>	67.5 <sup>b</sup>	238.6 <sup>b</sup>	245.4 <sup>c</sup>	110.6 <sup>c</sup>	114.1 <sup>c</sup>	53.2 <sup>a</sup>	54.6 <sup>a</sup>
T5	31.85 <sup>a</sup>	33.86 <sup>a</sup>	41.1 <sup>a</sup>	69.7 <sup>a</sup>	76.9 <sup>a</sup>	83.4 <sup>b</sup>	78.1 <sup>a</sup>	268.9 <sup>a</sup>	289.3 <sup>a</sup>	173.9 <sup>a</sup>	182.4 <sup>a</sup>	45.3 <sup>b</sup>	51.1 <sup>b</sup>

Therefore, N supply should be increased after anthesis to promote overall N<sub>up</sub> in the grain.

In summary, splitting applications of N could increase N absorbed by the plant and the grain, the NUE, the uptake of fertilizer-derived N, and reduce the consumption of N in the soil. Therefore, the 2:4:4 application ratio is the optimal N application method for DH661.

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