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Responses of nitrogen accumulation and translocation in five cytoplasmic hybrid rice cultivars

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Abstract: To study the difference among cytoplasm at the different nitrogen conditions, a research experiment was conducted using five different cytoplasmic male sterile (CMS) hybrid rice with nitrogen levels at N0, N1, N2, and N3; the nitrogen application rates were 0, 90, 180, and 270 kg/ha, from 2018 to 2019. Results showed that among tested cultivars of CMS hybrid rice, JW (J803A × Chenghui727) showed the highest yield in both years for the low nitrogen and high nitrogen treatments. The dry matter accumulation and translation of JW type in nutritive organs were higher than that of others during the low nitrogen level (N1). We concluded that the nutrient translocation within plants organs and dry biomass accumulation were highly dependent on CMS type and nitrogen application. This research indicates that selecting a rice cultivar with greater efficiency of nitrogen is favourable for raising the number of grains per panicle, grain yield, and nitrogen use efficiency. JW cytoplasm displayed great efficiency in low nitrogen, which is a potential cytoplasmic resource.

Keywords: cytoplasmic male infertility; chlorophyll fluorescence; nitrogen utilisation efficiency; grain weight; vegetative organs

Rice is one of the world's most important crops and is the staple diet of more than 2.7 billion people (Luo et al. 2021). To cope with the growing demand for food, by 2050, its production would have to be increased by 50% more than what is produced now (Bouwman et al. 2013). Currently, the genetic tools (male-sterile, maintainer, and restorer lines) essential to develop hybrid rice are available; parental lines adapted to rice-growing countries will also be available soon (Longkumar et al. 2020), which would significantly contribute to the increased global agricultural productivity.

Currently, in more than 90% of the hybrid rice area, hybrid rice derived from 1 source of cytosterility (WA) is planted, since this cultivar demonstrates the best stability with changing environmental conditions and is reasonably easy to restore (Virmani and Ilyas-Ahmed 2007). Hybrid rice has made a significant contribution to China's food security and remains a major source of top rice cultivars (Liao et al. 2021).

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However, hybrid rice production takes a long time, and the limited genetic resources available leave little space for improvement of rice potential (Xu et al. 2021). Over the last four years, International Rice Research Institute (IRRI) has continued to build new cytoplasmic male sterile (CMS), maintainer, and restorer lines in a cultivar of cytoplasmic and nuclear backgrounds (Turner and Bannin 2018). Recessive nuclear male-sterile lines could provide a reliable source of a female parent for hybrid seed development (unaffected by environmental changes), and they can be restored using virtually any germplasm containing the male-sterile mutation's wild type gene (Luo et al. 2021).

Nitrogen is the most abundant element on earth and is a required nutrient for the life cycle of plants. It is found in many organic compounds, including amino acids, proteins, coenzymes, nucleic acids, and chlorophyll (Kumar et al. 2021). The cereal crop's photosynthetic pigments highly depend on their nitrogen content. Previous studies report that increasing nitrogen concentrations results in improved photosystem II (PSII) potential activity and maximum quantum yield (Zhang et al. 2021b). Modern agriculture is highly dependent on nitrogen fertilisers since these have the strongest effect on increasing agricultural yield (Zhang et al. 2021a). Generally, proper fertiliser would lead to improved photosynthetic rate and agronomic traits, resulting in high dry matter accumulation and grain yield (Zhong et al. 2017), which ultimately increase high biomass accumulation (Bhattacharyya et al. 2012); However, excessive use of nitrogen fertiliser negatively impacts on rice and results in lower grain yield, photosynthetic rate, and dry matter accumulation (Xu et al. 2021). The agricultural sector of Asia is facing many issues related to nitrogen application in rice fields, including excessive usage of fertiliser, low rice plant utilisation rate, and high wastage rate (Zhang et al. 2020a). Therefore, it is important to investigate the required nitrogen fertiliser application level, which can reduce not only nitrogen fertiliser loss but also nitrogen fertiliser contamination of soil and the environment. The most common types of nitrogen that plant roots can absorb from the soil are ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N), and amino acids (Amisu 2021). Studies claim that the nitrogen assimilation ability and use efficiency of indica and japonica rice (*Oryza sativa* L.) sub-species differ (Kabange et al. 2020). Comparatively, indica has a higher ability to absorb nitrogen than japonica rice cultivars (Hu et al. 2015). Furthermore, different genotypes of indica rice cultivars were found to have different nitrogen uptake rates, grain yields, and nitrogen accumulation levels under the same nitrogen treatments in field experiments (Yang et al. 2018). Increased nitrogen use efficiency is a long-standing problem for indica rice cultivation, which is complicated by differences in nitrogen uptake characteristics of different indica rice cultivars (Yi et al. 2019).

Although many studies have been conducted to assess the importance of different forms of nitrogen uptake and utilisation in the context of southern Chinese hybrid rice (Yi et al. 2019), corresponding data are scarce in the context of wild abortive CMS (CMS-WA) hybrid rice crossed with wild hybrid rice type. To solve such problems related to rice planting and agricultural needs, in this study, we report different nitrogen fertilisation levels and their impacts on five cultivars of CMS-hybrid rice in the Sichuan basin area for the first time. The main objective of this study is to evaluate the effect of nitrogen accumulation and translocation at different nitrogen levels on rice plant growth, yield, yield traits, dry biomass accumulation, and nitrogen use efficiency of different cytoplasmic hybrid rice cultivars and their impact on photosynthetic pigments and photosynthesis rate. This will enable more efficient use of nitrogen and its components in the management of CMS-hybrid rice crops grown under field and pot conditions.

MATERIAL AND METHODS

Study area. The experiment was carried in the experimental base of Rice Research Institute of Southwest University of Science and Technology, Mianyang, from 2018 to 2019. Mianyang (104.73°E, 31.47°N), located at the middle and upper reaches of Fujiang River, is a typical subtropical monsoon climate, with an average annual temperature of 14.7–17.3 °C, a frost-free season of 252–300 days, and average annual precipitation of 826–1 417 mm, according to the weather records from 1984 to 2019. The field has typical clay-loam soil, with a bulk density of 1.29 g/cm^3 and the organic content content of 16.59 g/kg; the basic fertility of the experiment field was 1.98 g/kg total nitrogen, 80.3 mg/kg available nitrogen, 43.3 mg/kg available phosphorus, and 76.2 mg/kg available potassium. The local farmers of this area use about 180 kg/ha nitrogen fertiliser in rice fields.

Experimental design. Field design was carried out as a randomised complete block design with

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five different CMS hybrid rice cultivars: J803A × Chenghui 727, W803A × Chenghui 727, K803A × Chenghui 727, G803A × Chenghui 727, and Y803A × Chenghui 727, referred to as JW, W, K, G, and Y, respectively. These 5 hybrid rice were the same in the nuclear but different in CMS. The experimental design comprised four nitrogen levels: N0, N1, N2, and N3; the nitrogen application rates were 0, 90, 180, and 270 kg N/ha, respectively. Rice seeds were cultivated from March to September 2018, 2019. The test plot was 7-m long and 7-m wide, and the planting area of each plot was 3.5 m × 7 m, where 20 rows were planted, with 20 plants in each row and a planting size of 33.3 cm × 16.7 cm. There were three replications for each nitrogen fertiliser treatment. 20 plots were used for all treatments, and each plot was separated by a cement ridge 5-cm high and 30-cm thick to prevent the fertiliser from running water. Nitrogen (N), phosphorous (P), and potassium (K) applied for each plot were the same with different treatments of N, 38.7 kg P/ha and 99.6 kg K/ha, respectively. Nitrogen fertiliser (urea) was applied as the base (13), tiller (29), and panicle fertiliser (51) in the ratio of 5:3:2; potassium fertiliser (potassium chloride) was applied as the base (13) and panicle fertiliser (51) in the ratio of 1:1; phosphorus fertiliser (calcium superphosphate) was applied as base fertiliser (13) along with soil tillage before transplanting rice.

Samples and measurement

Sample collection. Fresh plant samples of all treatments were collected for morphological measurements. Three rice plants were randomly selected from five sampling points at the full heading (59) and maturity (89) stages per year. For dry weight analysis, samples dried at 105 °C for 30 min was mainly used for electrothermal de-enzyming, and then dried at 80 °C. The dry straw was divided into three parts: stalk, leave, and panicle.

Measurements of plant yield traits. Total 100 plants were collected from each plot for the measurements of yield and yield components, such as 1 000-grain weight, grain number per panicle, and productive tillers were calculated. The seed setting rate was measured by manually separating filled and unfilled grains of the panicle. To determine the grain yield per plant, a single plant's filled grains were collected and dried at 50 °C. For the 1 000-grain weight measurements, filled grains were chosen at random.

Measurements of plant nitrogen content

$$\text{Agronomic efficiency, AE (kg grain/kg)} = \frac{\text{grain yield in nitrogen application area} - \text{grain yield in nitrogen blank area}}{\text{nitrogen application amount}}$$

$$\text{Recovery efficiency, RE (\%)} = \frac{\text{nitrogen uptake by plants in nitrogen application area} - \text{nitrogen uptake by plants in nitrogen blank area}}{\text{nitrogen application amount}} \times 100\%$$

$$\text{Physiological efficiency, PE (kg grain/kg)} = \frac{\text{grain yield in nitrogen application area} - \text{grain yield in nitrogen blank area}}{\text{nitrogen uptake by plants in nitrogen application area} - \text{nitrogen uptake by plants in nitrogen blank area}} \times 100\%$$

Determination of chlorophyll content. Chlorophyll *a* and *b* content was determined by the Lichtenthaler and Wellburn (1983) method, using plants of 10 and 30 days after rice heading, three leaves (basal, central, and top) from each plant (3 replicate) cut at the basal part of the petioles, collected, labelled and placed in polyethylene zip-lock bags. Then placed on ice and kept in the dark while transported to the laboratory for processing. Collected leaves were blended with a homogeniser, placed in a 25 mL glass vial then 100 mg leaves homogenate added with 10 mL of 95.5% acetone. The glass vials were sealed with parafilm to prevent evaporation then stored at 4 °C for 48 h. The chl *a* and chl *b* concentrations were measured using an ultraviolet-visible (UV-Vis) spectrophotometer (Alpha-1506, Lab-Spectrum Instruments Co., Ltd., China) at 646 nm and 663 nm wavelengths and calculations were done as follows:

$$\text{Chl } a = 12.21\text{A663} - 2.81\text{A646}$$

$$\text{Chl } b = 20.13\text{A646} - 5.03\text{A663}$$

where: Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; A646 and A663 – absorbance values of the extraction solution at wavelengths of 646 nm and 663 nm, respectively.

Determination of chlorophyll fluorescence. The FM/S^2 instrument was used for the measurement of chlorophyll fluorescence. After 30 days of rice heading, three leaves (basal, central, and top) from each plant (3 replicate) were selected for the measurement of each cultivar and treatment. First, dark treatment was carried out on the leaves for 20 min, and then following parameters were measured: F_v/F_m (the maximum photochemical quantum yield of PSII), F_o (fixed fluorescence, is the reaction of PSII; the fluorescence yield when the centre is completely open), and F_m (the maximum fluorescence yield when the PSII reaction centre is completely closed).

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Statistical analysis. Statistical analysis was performed to check the homogeneity of the variances, and the data were analysed using the analysis of variance (SPSS 16.0). Differences among treatment means were determined using the least significant difference (*LSD*) at the 0.05 and 0.01 probability levels. Microsoft Excel 2016 (Redmond, USA) was employed for data processing and graphics.

RESULTS

Yield and its components under different nitrogen levels. The response of different cytoplasmic sources to nitrogen fertiliser input was significantly different (Table 1), and the analysis of the coefficient of variation at each level showed that the cytoplasmic effect varied the most under low nitrogen treatment. Based on the two-year data, under the low nitrogen (N1) level, the yield of the JW cytoplasmic combination was relatively high. The yield of the JW cytoplasmic combination increased by 10.8% (2018), 6.2% (2019) compared lowest yield found in Y-type (2018) and G-type (2019). Under the high nitrogen (N3) condition, the yield of the JW-type cytoplasmic combination was higher than that of other cytoplasmic combinations, by 7.1% (2018) and 7% (2019), respectively. To clarify the difference in response of cytoplasm to different nitrogen fertiliser levels in terms of yield traits, the differences in yield traits among cytoplasm were compared under different nitrogen fertiliser levels (Table 1). At low nitrogen (N1) conditions, the W cytoplasmic combination showed a higher 1 000-grain weight for two consecutive years, which was 3.4% higher than other cytoplasmic combinations. Under high nitrogen (N3) level, W-type cytoplasm had a significant

Table 1. Grain yield, 1 000-grain weight, grains per panicle, and productive tillers of five cytoplasmic male sterile (CMS) hybrid rice grown in 2018 and 2019

Treatment	2018				2019			
	yield (t/ha)	TGW (g)	GP (grain/panicle)	PT (× 10^3/ha)	yield (t/ha)	TGW (g)	GP (grain/panicle)	PT (× 10^3/ha)
N0-JW	7.66^{a}	28.77^{b}	157.78^{c}	1 327^{b}	7.21^{a}	29.28^{b}	181.07^{a}	1 020^{b}
N0-W	6.99^{b}	28.42^{b}	153.22^{c}	1 332^{b}	6.89^{b}	29.92^{ab}	167.88^{b}	1 080^{ab}
N0-G	7.39^{ab}	28.74^{b}	173.82^{b}	1 332^{b}	6.52^{c}	29.64^{ab}	169.67^{b}	984^{c}
N0-K	7.48^{ab}	28.59^{b}	174.85^{b}	1 354^{a}	6.97^{b}	29.07^{b}	158.48^{c}	1 116^{a}
N0-Y	7.55^{a}	30.02^{a}	182.94^{a}	1 342^{ab}	7.07^{ab}	30.31^{a}	179.56^{a}	1 080^{ab}
N1-JW	8.91^{a}	29.73^{b}	164.49^{b}	1 716^{a}	9.02^{a}	31.24^{b}	150.26^{c}	1 608^{ab}
N1-W	8.66^{b}	30.76^{a}	157.50^{c}	1 656^{b}	8.76^{b}	32.35^{a}	159.64^{b}	1 704^{a}
N1-G	8.08^{c}	30.23^{ab}	171.63^{a}	1 716^{a}	8.46^{c}	31.69^{ab}	151.82^{c}	1 440^{cd}
N1-K	8.57^{b}	30.42^{ab}	173.37^{a}	1 620^{c}	8.94^{ab}	31.81^{ab}	166.58^{b}	1 512^{bc}
N1-Y	7.94^{c}	29.71^{b}	175.70^{a}	1 656^{b}	8.74^{b}	31.48^{b}	174.16^{a}	1 332^{d}
N2-JW	10.01^{a}	30.05^{b}	169.75^{b}	1 752^{a}	9.84^{ab}	32.16^{a}	166.75^{b}	1 464^{b}
N2-W	9.19^{b}	30.38^{ab}	171.63^{b}	1 680^{b}	9.64^{b}	32.29^{a}	170.12^{ab}	1 500^{c}
N2-G	9.26^{b}	30.92^{a}	184.30^{a}	1 632^{c}	9.34^{c}	30.88^{b}	164.85^{b}	1 680^{a}
N2-K	9.06^{c}	30.6^{ab}	183.06^{a}	1 680^{b}	10.18^{a}	31.73^{ab}	172.35^{ab}	1 632^{ab}
N2-Y	9.00^{c}	29.92^{b}	182.30^{a}	1 692^{b}	9.96^{ab}	31.78^{ab}	177.12^{a}	1 584^{bc}
N3-JW	10.03^{a}	29.68^{a}	165.12^{c}	1 884^{a}	10.10^{a}	30.43^{b}	166.01^{b}	1 620^{a}
N3-W	9.31^{c}	29.72^{a}	173.52^{b}	1 848^{b}	9.57^{b}	31.91^{a}	168.89^{b}	1 716^{a}
N3-G	9.32^{c}	29.56^{a}	167.29^{c}	1 800^{c}	9.39^{b}	31.69^{ab}	167.54^{b}	1 596^{b}
N3-K	9.79^{b}	29.67^{a}	177.94^{ab}	1 812^{c}	10.05^{a}	30.79^{b}	168.39^{b}	1 620^{ab}
N3-Y	9.35^{c}	28.69^{b}	182.34^{a}	1 752^{d}	9.89^{ab}	31.93^{a}	173.64^{a}	1 692^{a}

TGW – 1 000 grain geight; GP – grains per panicle; PT – productive tillers; N0 – 0, N1 – 90, N2 – 180, N3 – 270 kg N/ha; JW – J803A × Chenghui 727; W – W803A × Chenghui 727; K – K803A × Chenghui 727; G – G803A × Chenghui 727; Y – Y803A × Chenghui 727. Within the same column, cultivar, and year, means not sharing a letter were significantly different at the *P* = 0.05 probability level according to the Tukey *LSD* (least significant difference) test

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increase of 3.4% as compared with other cytoplasmic combinations.

Under different nitrogen treatment levels, the yield of the JW-type cytoplasmic combination was at a higher level, and when treated with low nitrogen, the advantages were more significant. Under high nitrogen treatment, the yield of JW-type cytoplasmic rice was also at a higher level.

Differences in dry matter accumulation and distribution of vegetative organs. There were significant differences in the accumulation and distribution of dry matter in different cytoplasmic plants at different stages of rice growth (Figure 1). In 2018, when the JW-type cytoplasm was treated with low nitrogen (N1), dry matter accumulation in the stalk, leaf and panicle at the full heading increased by 11.7% compared with other cytoplasmic combinations. In 2019, similar to the full heading, dry matter accumulation in the stalk, leaf and panicle of the W-type increased as compared with other cytoplasmic combinations. In 2018, when treated with high nitrogen (N3), the dry matter accumulation in the stalk, leaf and panicle of Y and K-types cytoplasm at the full heading increased significantly as compared with others. In 2019, similar to the full heading, dry matter accumulation in the stalk, leaf and panicle of the JW-type increased significantly as compared with other cytoplasmic combinations. The results showed that under low nitrogen treatment, the dry matter accumulation of JW cytoplasmic rice was higher during different growth and development stages. JW and K-types were processed under high nitrogen (N3) conditions, in which it is easy to obtain higher dry matter accumulation to achieve a higher yield.

At the maturity stage, the dry matter of rice plants accumulated mostly in the stalk. After the full heading stage, till the maturity stage, the dry matter mainly accumulated in the leaf, and the proportion increased significantly. In 2018, under the low nitrogen (N1) treatment, the accumulation of dry matter in the stalk, leaf and panicle of JW cytoplasm at the maturity stage was 18.5% higher than that of other types, similar results were obtained in 2019. In 2018, under high nitrogen (N3) treatment, the accumulation of dry matter in the stalk, leaf and panicle of K-type cytoplasm at the maturity stage increased by 15.5% compared with another cytoplasm. In 2019, similar to the maturity stage, dry matter accumulation in the stalk, leaf and panicle of the Y and JW-types increased, but K-type cytoplasmic was at a relatively low level due to environmental factors.

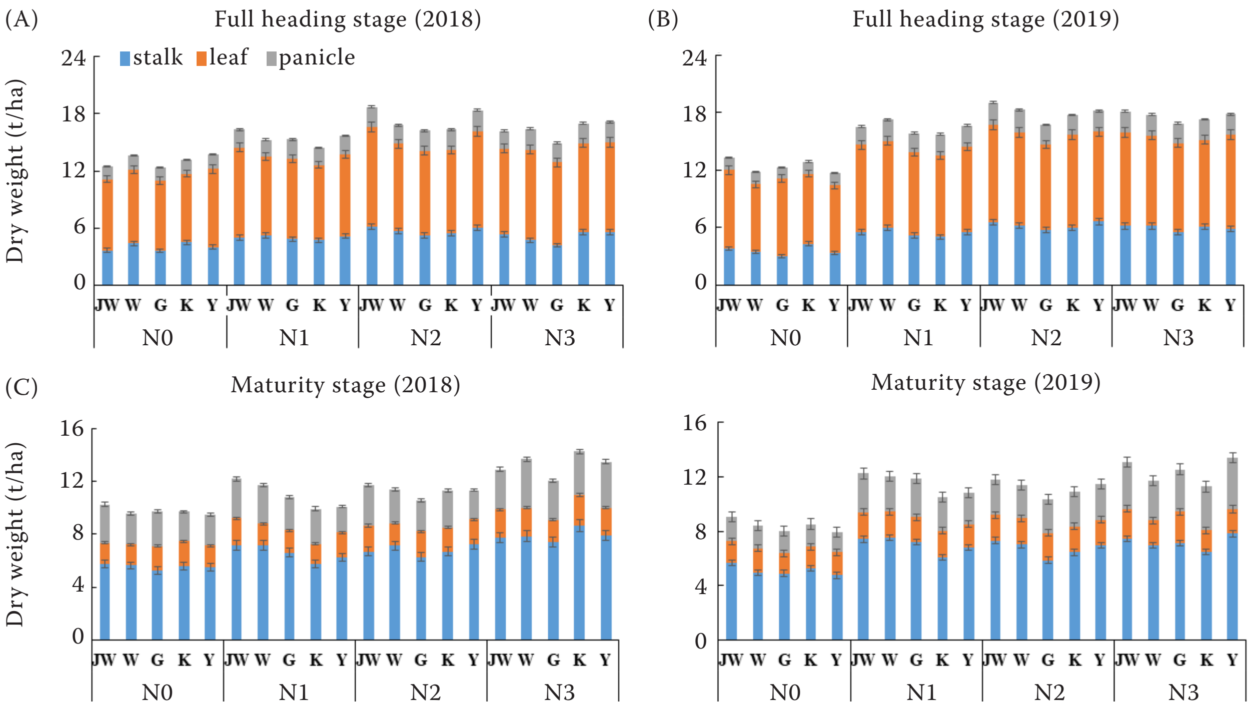

Figure 1. Effects of different nitrogen fertiliser treatments on the accumulation and distribution of different cytoplasmic rice combinations (2018, 2019). (A) and (B) showed full heading stage (59) of 2018–2019, while (C) and (D) showed maturity stage (89) 2018–2019. Error bars indicate standard errors of the replicates (n = 3). N0 – 0, N1 – 90, N2 – 180, N3 – 270 kg N/ha; JW – J803A × Chenghui 727; W – W803A × Chenghui 727; K – K803A × Chenghui 727; G – G803A × Chenghui 727; Y – Y803A × Chenghui 727

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Nitrogen use efficiency. Significant differences were observed in the cytoplasm of hybrid rice's nitrogen absorption, utilisation, and transport efficiency under different nitrogen fertiliser levels (Table 2). The results showed that under the condition of low nitrogen (N1), these indicators of G-type cytoplasmic rice were all higher than that of others. From the normal nitrogen (N2) treatment to the high nitrogen (N3) treatment, these indicators showed a decreasing trend. Under the condition of high nitrogen (N3), the K-type cytoplasmic nitrogen utilisation efficiency indexes indicated higher tolerance to high nitrogen. Compared with other cytoplasmic combinations, the agronomic and physiological utilisation rate of nitrogen fertiliser of K-type cytoplasm increased by 13%, and 2.9%, respectively. Comprehensive analysis showed that under low nitrogen levels, the absorption and utilisation of G-type nitrogen were relatively high. With the increase of nitrogen fertiliser application, compared with other cytoplasmic combinations, K-type cytoplasmic rice displayed relatively high values for nitrogen absorption, transport, and utilisation indicators.

Response of chlorophyll *a* and *b* content to different nitrogen treatments. Chlorophyll is the main pigment for the photosynthesis of plants. Therefore, the quantitative analysis of chlorophyll is extremely important. Table 3 shows that under the nitrogen fertiliser treatment, there are certain differences in the chlorophyll *a* and *b* content between the different cytoplasm. It is also one of the important reasons for the difference in the yield of each cytoplasmic combination. Under low nitrogen (N1) conditions, the JW-type cytoplasm showed the maximum chlorophyll *a* and *b* content. Compared with other cytoplasmic combinations, the chlo-

Table 2. The response of sterile cytoplasm to nitrogen fertiliser in nitrogen absorption and utilisation

	2018			2019		
	agronomic utilisation of N	N absorption and utilisation rate	N physiological utilisation rate	agronomic utilisation of N	N absorption and utilisation rate	N physiological utilisation rate
	(kg grain/kg N)					
N0-JW	–	–	–	–	–	–
N0-W	–	–	–	–	–	–
N0-G	–	–	–	–	–	–
N0-K	–	–	–	–	–	–
N0-Y	–	–	–	–	–	–
N1-JW	13.64^{b}	20.05^{a}	58.00^{ab}	19.19^{bc}	37.52^{c}	51.12^{a}
N1-W	18.61^{a}	12.02^{b}	55.63^{b}	20.77^{ab}	47.92^{a}	43.33^{c}
N1-G	7.75^{c}	8.53^{c}	60.46^{a}	15.91^{d}	49.52^{a}	52.11^{a}
N1-K	12.11^{b}	21.50^{a}	56.41^{b}	22.77^{a}	39.66^{b}	50.39^{ab}
N1-Y	4.31^{d}	12.62^{b}	34.14^{c}	18.55^{c}	38.3^{bc}	48.49^{b}
N2-JW	13.84^{a}	38.79^{a}	35.70^{c}	14.59^{c}	33.61^{c}	43.41^{b}
N2-W	12.26^{b}	19.94^{b}	61.54^{a}	15.32^{bc}	37.83^{b}	40.51^{b}
N2-G	10.42^{c}	17.27^{c}	60.39^{a}	15.63^{bc}	31.09^{d}	50.27^{a}
N2-K	8.77^{d}	15.65^{d}	56.06^{ab}	17.84^{a}	33.58^{c}	53.12^{a}
N2-Y	8.04^{d}	15.84^{d}	50.74^{b}	16.07^{b}	39.17^{a}	41.05^{b}
N3-JW	8.75^{a}	26.80^{a}	32.64^{c}	10.71^{ab}	20.25^{b}	52.94^{a}
N3-W	8.62^{a}	25.49^{b}	33.80^{c}	9.93^{b}	19.27^{c}	51.52^{a}
N3-G	7.17^{b}	13.56^{e}	52.85^{a}	10.60^{ab}	20.40^{b}	51.99^{a}
N3-K	8.55^{a}	21.70^{c}	39.39^{b}	11.42^{a}	21.52^{a}	53.10^{a}
N3-Y	6.66^{b}	18.67^{d}	35.69^{bc}	10.46^{b}	20.11^{b}	52.01^{a}

Within the same column, cultivar, and year, means not sharing a letter were significantly different at the P = 0.05 probability level according to the Tukey *LSD* (least significant difference) test. N0 – 0, N1 – 90, N2 – 180, N3 – 270 kg N/ha; JW – J803A × Chenghui 727; W – W803A × Chenghui 727; K – K803A × Chenghui 727; G – G803A × Chenghui 727; Y – Y803A × Chenghui 727

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Table 3. The response of chlorophyll *a* and *b* towards nitrogen different treatments of five cytoplasmic male sterile (CMS) hybrid rice grown in the years 2018 and 2019

Treatment	Cultivar	2018		2019	
		Chl *a*	Chl *b*	Chl *a*	Chl *b*
N0	JW	2.56 ± 0.08[a]	0.79 ± 0.09[a]	2.58 ± 0.02[a]	1.07 ± 0.08[a]
	W	2.46 ± 0.05[ab]	0.68 ± 0.02[a]	2.53 ± 0.05[ab]	0.87 ± 0.07[b]
	G	2.16 ± 0.05[c]	0.48 ± 0.01[b]	2.38 ± 0.08[b]	0.82 ± 0.04[c]
	K	2.56 ± 0.05[a]	0.78 ± 0.09[a]	2.61 ± 0.07[a]	0.88 ± 0.08[b]
	Y	2.36 ± 0.04[b]	0.68 ± 0.01[a]	2.42 ± 0.04[b]	0.87 ± 0.08[b]
N1	JW	2.76 ± 0.04[a]	0.79 ± 0.03[a]	2.79 ± 0.08[a]	1.00 ± 0.05[a]
	W	2.48 ± 0.08[b]	0.70 ± 0.09[ab]	2.48 ± 0.07[b]	0.80 ± 0.04[c]
	G	2.24 ± 0.05[c]	0.59 ± 0.07[b]	2.37 ± 0.06[b]	0.71 ± 0.05[d]
	K	2.57 ± 0.09[b]	0.70 ± 0.01[ab]	2.69 ± 0.06[a]	0.87 ± 0.05[b]
	Y	2.47 ± 0.07[b]	0.69 ± 0.02[ab]	2.37 ± 0.05[b]	0.80 ± 0.08[c]
N2	JW	2.86 ± 0.05[a]	1.18 ± 0.06[a]	2.93 ± 0.06[a]	1.38 ± 0.06[a]
	W	2.36 ± 0.09[b]	0.88 ± 0.06[bc]	2.68 ± 0.05[b]	1.15 ± 0.04[b]
	G	2.26 ± 0.09[b]	0.79 ± 0.08[c]	2.49 ± 0.08[c]	0.89 ± 0.04[d]
	K	2.76 ± 0.07[a]	0.99 ± 0.03[b]	2.68 ± 0.05[b]	0.96 ± 0.07[c]
	Y	2.36 ± 0.03[b]	0.89 ± 0.07[bc]	2.55 ± 0.07[bc]	0.90 ± 0.08[d]
N3	JW	2.80 ± 0.07[a]	1.09 ± 0.02[a]	2.87 ± 0.08[a]	1.32 ± 0.06[a]
	W	2.64 ± 0.02[b]	0.89 ± 0.06[b]	2.78 ± 0.06[a]	0.88 ± 0.05[c]
	G	2.36 ± 0.02[c]	0.69 ± 0.07[c]	2.40 ± 0.09[b]	0.85 ± 0.05[c]
	K	2.71 ± 0.02[ab]	1.18 ± 0.06[a]	2.86 ± 0.04[a]	1.25 ± 0.08[b]
	Y	2.36 ± 0.04[c]	0.78 ± 0.05[bc]	2.43 ± 0.03[b]	0.86 ± 0.08[c]

Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*. All values represent mean ± standard deviation (n = 3) within the same column, cultivar, and year, means not sharing a letter were significantly different at the P = 0.05 probability level according to Tukey *LSD* (least significant difference) test. JW – J803A × Chenghui 727; W – W803A × Chenghui 727; K – K803A × Chenghui 727; G – G803A × Chenghui 727; Y – Y803A × Chenghui 727

rophyll *a* and chlorophyll *b* of the JW cytoplasm increased by 16.3% and 35.6%, respectively, under high nitrogen (N3) conditions.

The maximum quantum yield of PSII (F_v/F_m). In both years, the different nitrogen levels had a significant effect on the dynamics of the F_v/F_m. Figure 2 shows that the CMS cultivars displayed differences in all treatments. In 2018, JW and G-type CMS showed the highest F_v/F_m values in N1 treatment, which were, respectively, 10.7% and 19% higher than in the N0 application. In 2019, the overall mean of all treatments was higher than in 2018. JW and K type collectively showed higher F_v/F_m values in N3 treatments, which were, respectively, 22.6% and 25% higher than in N0 treatment. The F_v/F_m values of the different CMS cultivars were significantly different in two years, and the interaction effects between the nitrogen levels and cultivars were also significant.

DISCUSSION

According to Lin et al. (2020), hybrid progenies have better growth characteristics than parental lines. In this study, both year's outcomes in different nitrogen treatments were different, with higher values obtained in 2019. During both years, the yield of the JW cytoplasmic combination was relatively high under low nitrogen (N1) levels; the yield of the JW cytoplasmic combination increased by 10.8% (2018) and 6.2% (2019) as compared to other cytoplasmic combinations. The findings suggest that different CMS hybrids respond differently to low and high nitrogen treatments. JW combination favoured N1 level nitrogen and produced a higher yield than other CMS hybrids. The results are consistent with observations made by Chen et al. (2020), who found that nitrogen fertilisation affected positively and yield increased from 5.9 to 9.0 t/ha. Similarly, the find-

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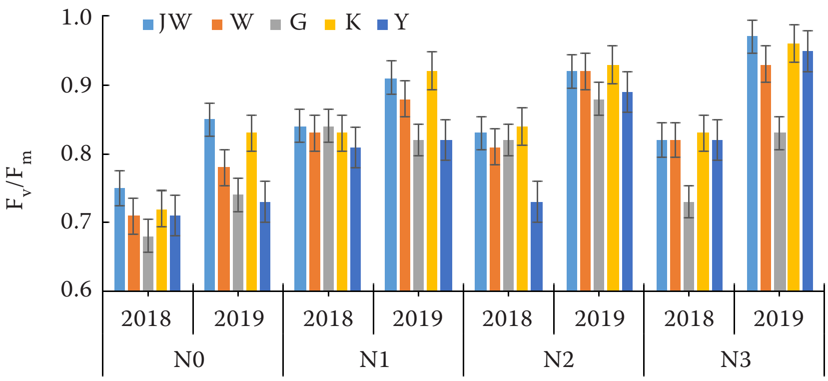

Figure 2. The maximum quantum yield of PSII (F_v/F_m) for the years 2018 and 2019. Error bars indicate standard errors of the replicates (*n* = 3). N0 – 0, N1 – 90, N2 – 180, N3 – 270 kg N/ha; JW – J803A × Chenghui 727; W – W803A × Chenghui 727; K – K803A × Chenghui 727; G – G803A × Chenghui 727; Y – Y803A × Chenghui 727

ings also showed a great increase in yield and yield traits for different nitrogen fertilisation treatments.

Many factors can affect the nitrogen uptake and utilisation rate of the rice plant, among which the nitrogen application time and quantity are most important. The nitrogen use efficiency (NUE) measures the response of yield to increase the nitrogen rate (Miao et al. 2016). The agronomic utilisation rate and the physiological utilisation rate of nitrogen fertiliser of G cytoplasmic rice were higher under low nitrogen (N1). These indicators decreased from the usual nitrogen (N2), and high nitrogen (N3) treatments. With high nitrogen (N3), the K-type cytoplasmic nitrogen utilisation efficiency-related indexes indicated that it is more tolerant to high nitrogen. Compared with other cytoplasmic combinations, the agronomic utilisation rate, and the physiological utilisation rate of nitrogen fertiliser, K-type cytoplasm increased by 13%, and 2.9%. Under low nitrogen levels, the absorption and utilisation of G-type nitrogen were relatively high. With increased application of nitrogen fertiliser, compared with other cytoplasmic combinations, K-type cytoplasmic rice nitrogen absorption and transportation were relatively high.

The nutrient use efficient genotypes are those having the ability to produce sufficient biomass under limited nutrient availability (Chatzistathis and Therios 2013). Similarly, in this study, the JW-type rice had the highest dry biomass accumulation at low nitrogen input, while as nitrogen fertilisation increased, it reduced significantly. Conversely, other cultivars showed increased dry biomass accumulation at higher nitrogen treatments. Our study is consistent with the study of Zhang et al. (2020), in which the authors concluded that appropriate nitrogen could improve photosynthesis, which results in higher biomass accumulation.

The amount of nitrogen applied affects the photosynthesis of plants (Moe et al. 2019). Appropriate nitrogen management can significantly increase the development of both photosynthetic pigment and photosynthesis rate (Peng et al. 2021). Nitrogen application had a great effect on photosynthetic pigment chlorophyll *a* and *b*. The total chlorophyll content increased under a high nitrogen supply in all five CMS. In both years, for all CMS combinations, the change in chlorophyll pigment was significantly correlated with an increase in nitrogen concentration. Under low nitrogen (N1) conditions, the JW-type CMS showed the maximum chlorophyll *a* and *b* contents. Compared with other cytoplasmic combinations, the chlorophyll *a* and *b* of the JW cytoplasm increased by 16.3% and 35.6%, respectively, under high nitrogen (N3) conditions. Pan et al. (2016), reported similar results it was concluded that an increase in nitrogen application increases photosynthetic ability, which positively affects plant biomass and yield quality.

Our findings led to the conclusion that under low nitrogen treatment, the dry matter accumulation of JW cytoplasmic rice was higher in different growth and development stages. Thus, it shows that different cultivars of cytoplasmic rice have different nitrogen fertilisation requirements. Thus, as a mean for all CMS combinations, 180 kg/ha is suggested as the most suitable dose of nitrogen fertiliser, which efficiently increases yield, biomass accumulation, nitrogen use efficiency, development of better photosynthetic pigment and helps in the photosynthesis process of JW CMS combination. Apart from nitrogen use efficiency, more research is needed for phosphorus and potassium requirements for such CMS combinations.

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