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Full Length Research Paper

High/low nitrogen adapted hybrid of rice cultivars and their physiological responses

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Seventy (70) selective bred hybrid of rice combinations were treated with three levels of pure nitrogen ($N_{1/2}$, 20.0mg/l; N_1, 40.0mg/l; N_2, 80.0mg/l) for two weeks for indentifying the adaptation of nitrogen. They were divided into six types with the cluster analysis as flowers: Type 1 with good adaptability of different concentrations of nitrogen, their ratio of root and stem (R/S ratio) was significantly increased under $N_{1/2}$ and N_2 treatment; but type 6 with sensitivity to those environments, the R/S ratio was significantly decreased under corresponding treatments. Other four types were lying in the middle with moderate adaptability of nitrogen. Furthermore, five varieties were selected from each of the mentioned six types and then grew in N-free conditions to investigate their performance on lack of N. The results showed that type 1, the dry weight of the materials originally growing in N_2 and $N_{1/2}$ conditions were both decreased, which had the lowest adaptability to N-free conditions; type 3, those of the materials growing similar conditions were both increased, which had better adaptability to N-free conditions. On the whole physiologically, the rice materials with good adaption of N were due to higher nitrate reductase activity (NRA) at low nitrogen conditions, more chlorophyll content and soluble protein in high nitrogen conditions, and the Rubisco mobilized in N-free conditions as well.

Key words: Rice, ratio of root and stem, SPAD, nitrogen, adaptation.

INTRODUCTION

Nitrogen is one of the most important nutrients for plant growth and a major factor that limits agricultural yields (Brodbent and Detta, 1987; Elena et al., 2008; Lawlor 2002; Dai et al., 2009). At present, both production and consumption of nitrogen fertilizer occupy the first place in the agricultural production all over the world. China is one of the countries that consume most nitrogen fertilizer and the consumption per unit area is also above the world average, but the availability of nitrogen fertilizer is only 30 to 41% (Zhu and Wen, 1992). The pollution of eutrophication in surrounding waters is increasingly aggravated as a result of the waste of excessive nitrogen in farmlands (Spietz, 2009). Therefore, the selection -breeding of nitrogen-efficient and low nitrogen-tolerant cultivars is one major way to increase the availability of nitrogen, to reduce environmental pollution and the waste of resources and to achieve the sustainable development of agriculture. It has also become one of the hot spots in crop science research in recent years.

There have been many reports on nitrogen absorption in crops since Harvey (1939) first reported the difference in nitrogen absorption between different cultivars of corns (Inthapanya and Sipaseuth, 2000; Koutroubas and Ntanos, 2003; Piao et al., 2001; Piao et al., 2003; Fukushima et al., 2011). However, current studies of the nitrogen nutrition of rice are mainly focused on the aerial parts, such as leaves, in which the absorption and utilization efficiency of the nitrogen nutrition, the effective state and applications of nitrogen fertilizer and the relationship between its application amount and production, quality and related physiology are investigated (Braoadbent and Detta, 1987; Samonte et al., 2006; Datta and Braoadbent, 1990; Wu and Tiao, 1995; Zeng et al., 2007). However, under the long-term domestication of artificial cultivation, the response of rice to nitrogen is a complicated dynamic process; in addition to photosynthesis, the root system responds to the absorption of nitrogen

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significantly (Song and Li, 2004; Cassman et al., 1998). The studies on the adaptability of different hybrid rice materials to nitrogen and the influence of different levels of nitrogen on the tolerance of rice in N-free conditions are particularly rare. In this paper, the adaptability of 70 selective bred hybrid of rice combinations to high and low nitrogen during the seedling period was screened and identified. However, six rice typical materials which showed different adaptability to different levels of nitrogen were selected to investigate the physiological basis of cultivar differences, and the selection indexes of adaptability were determined in the different levels of nitrogen of the rice materials with sound aerial and underground parts in the hope of providing important technical references for field mass screening of nitrogen -adapted rice materials and rational fertilization.

MATERIALS AND METHODS

Experimental materials

Seventy (70) hybrid of rice combinations (No. as 801-870) selectively bred in Lu Chuangen's team in Jiangsu Academy of Agricultural Sciences were selected as test materials. The seeds were sterilized with 75% alcohol for 5 min and with 0.1% $HgCl_2$ for 15 min, and then washed with distilled water for three times. The germination acceleration was conducted after the seeds had been soaked for 48 h. After 90% of seeds germinated about 48 h later, they were sowed in the nursery of the Food Crops Research Institute of Jiangsu Academy of Agricultural Sciences at May, 2008 and May, 2009. Seedlings at the six-leaf stage were taken for water cultivation.

Treatment methods

Treatment of high/low nitrogen

IRRI conventional nutrient solution for rice was adopted as the basic nutrient solution for water cultivation. The turnover boxes of $15\times40\times60$ cm^3 were used as containers, each containing 20 L nutrient solution at a time. The rice seedlings were fixed with foam with a row space of 6×8 cm^2. During water cultivation, they were cultivated with conventional nutrient solution for one week to ensure a relatively uniform nitrogen condition. The nutrient solution was replaced every three days, then three different levels of nitrogen were used for the cultivation, that is, 1/2 times of standard nitrogen level (20.0 mg N was added into basic medium, $N_{1/2}$), 1 time of standard nitrogen level (40.0 mg N was added into basic medium, N_1) and 2 times of standard nitrogen level (80.0 mg N was added into basic medium, N_2). 300 plants were cultivated for each material, 30 plants of even growth were selected for each treatment, and each treatment was done in triplicate.

After two weeks of water cultivation, growing materials under the same treatment were divided into two groups. The plants were taken from each group and their dry weight and SPAD value were measured. The weight ratio of root and stem was calculated and the top second leaf was cut off at the leaf sheath from the plants, wrapped with the foil, labelled, quickly frozen in liquid nitrogen and stored at -80℃ in the refrigerator and their physiological indexes such as enzyme activity were measured.

N-free treatment

Another group of the rice materials, which were cultivated for two weeks with different levels of nitrogen, were cultivated for another two weeks with N-free conventional nutrient solution (conventional nutrient solution in which the nitrogen was directly disposed). The physiological indexes of rice were measured in parallel after the N-free treatment.

Measurements

Six materials with different adaptability to different levels of nitrogen were selected. The uniformed growing plants from each material were selected for physiological response research. The plants were fixed at 105℃ for 15 min, and then dried to the constant weight at 80℃. The total dry weight and root dry weight of rice materials were measured with three plants and used to calculate the ratio of root and stem. The chlorophyll content in the leaves of three linked plants was measured by SPAD-502 *in vivo* chlorophyll meter made in Japan as the SPAD value. Nitrate reductase activity (NRA) was measured with three plants in accordance with the methods proposed by Huang (1999). The methods of Makino were used for the extraction of Rubisco with three plants (Makino et al., 1986). The content of soluble protein was measured with 3 plants in accordance with the methods proposed by Bradford (1976).

Data analysis

The correlation analysis and cluster analysis were conducted with SPSS 17.0. The correlation was Pearson's correlation and the cluster was K-means cluster. The contents of Rubisco were analyzed with Bandscan 5.0 software.

RESULTS

Responses and cluster analysis of 70 different rice materials in different nitrogen concentrations

As the main organ of nutrient absorption and transportation in plants, the root system is the direct user of the soil nutrients and the important contributor of the yield. Its function is closely related to its morphological characteristics and physiological mechanism, and is under the dual influence of gene control and environmental factors. Generally speaking, the root system with a well developed morphology usually has good physiological characteristics. That is, there is consistency between the morphological development and physiological characteristics of the root system. Wu and Tao (1995) held that, the good morphological development of the root system increased the surface of its contact with nitrogen and its higher oxidization and reduction abilities and energy consumption provided sufficient energy for the rice's root system to absorb nitrogen, thus, promoting its efficient nitrogen absorption and the ratio of root and stem (R/S ratio, RSR) was one of the most sensitive physiological indexes responsive to nitrogen in the seedling stage. In this paper, after two weeks of nitrogen treatment with different concentrations, the R/S ratio of different experimental rice materials showed different changes. According to the percentage of the difference between the R/S ratio in different nitrogen concentrations (that is $N_{1/2}$ or N_2) with that in N_1 concentration in the R/S

Table 1. The cluster analysis of the RSR of 70 rice materials treated by different N concentration.

Cluster	Number	Material	$(RSR_{N1/2}-RSR_{N1})/RSR_{N1}\times100$ (%) Changes of the RSR of $N_{1/2-}$		$(RSR_{N2}-RSR_{N1})/RSR_{N1}\times100\%$(%)C hanges of the RSR of N_2	
			Mean	Standard deviation	Mean	Standard deviation
1	7	809,811,819,825,850,860, 863	13.54^{Aa}	0.10068	13.04^{Bb}	0.02239
2	20	804,805,808,810,816,820, 822,826,832,833,834,837, 839,842,849,856,861,866, 867,869	2.16^{Bb}	0.04434	2.2^{Dd}	0.03779
3	14	801,814,818,821,823,828, 836,843,845,847,848,858, 864,870	1.00^{Cc}	0.03003	18.52^{Aa}	0.06296
4	9	806,824,831,835,840,844, 846,851,855	-8.92^{Dd}	0.03310	11.59^{Cc}	0.02371
5	10	802,803,807,812,815,829, 838,854,862,868	-12.88^{Ee}	0.04065	$- 3.66^{Ee}$	0.03873
6	10	813,817,827,830,841,852, 853,857,859,865	-22.70^{Ff}	0.04521	-15.88^{Ff}	0.06662
Total	70		4.06	0.11601	4.45	0.12040

A-F shows very significant differences ($P < 0.01$); a-f shows significant differences ($P < 0.05$)

ratio, the cluster screening was conducted with the K-means method and the typing was conducted at the threshold value of about 5. The results showed as in Table 1 that, the difference between different types of numerical values was highly significant. There were mainly 6 types: type 1, the R/S ratio significant increased under $N_{1/2}$ or N_2 treatment; type 2, the R/S ratio did not show obvious change under $N_{1/2}$ or N_2 treatment (2.2 and 2.16%); type 3, the R/S ratio significantly increased under $N_{1/2}$ treatment, but did not show obvious change in N_2; type 4, the R/S ratio showed a significant increase under $N_{1/2}$ treatment, but also a significant decrease in N_2 treatment; type 5, the R/S ratio did not show obvious change under $N_{1/2}$ treatment, but it significantly decreased under N_2 treatment; and type 6, the R/S ratio had a significant decrease in $N_{1/2}$ or N_2 treatment (-15.88 and -20.70%). Thus, it could be seen that different rice materials showed different responses to different levels of nitrogen in the seedling stage.

Responses to N-free treatment cluster analysis of 30 rice materials after N-free treatment

Plants have significant genotypic differences in their abilities to absorb and utilize different nutrients and adaptabilities to environment, so it is essential to explore and use the genetic specificity of the plants to improve their nutritional trait and to select species or varieties suitable for specific soil environments, thus, enabling the plants adapt to the environment. Therefore, the cultivation of varieties with a strong "buffering" capacity to the changes in nitrogen supplies or adjustment in fertilization methods, can meet the demand of rice for nitrogen. There is an obvious positive correlation between the chlorophyll content in the leaf and its nitrogen content as well as the level of nitrogen supplies. The nitrogen content in the leaf can sensitively reflect the dynamic demand of the plant for nitrogen; the chlorophyll meter (SPAD) provides a simple, fast and damage-free method to measure the nitrogen content. A total of 30 materials, 5 materials from each of 6 types primarily screened by the R/S ratio, were further selected in this study to receive 2 weeks of N-free treatment and the changes in the leaf's R/S ratio, SPAD and dry weight of individual plants were measured to examine the responses of rice materials in different nitrogen concentrations to N-free conditions. The change of the ratio of the individual plant's dry weight in different nitrogen concentrations to that of N_1 concentration and the leaf's SPAD after treatment were employed together in the screening with K-means clustering and the typing was conducted when the threshold value was about 5 (Table 2). The experimental materials could be divided into three types, where there were no obvious differences in R/S ratio and SPAD changes but significant changes in dry weight: type 1, the dry weight of the materials growing in $N_{1/2}$, N_2 environment decreased, which had the worst adaptability to N-free conditions in either high or low nitrogen

Table 2. The cluster analysis of the SPAD, R/S ratio and dry weight of different rice materials grown in different N concentration after N-free treatment.

Cluster	Number	Material	SPAD after N-free treatment	R/S ratio after N- free treatment		Changes of the dry weight of $N_{1/2}$ after N-free treatment	Changes of the dry weight of N_2 after N-Free treatment
				(R/S $_{N1/2}$)/ (R/S $_{N1}$)	(R/S $_{N2}$)/ (R/S $_{N1}$)	(Dry weight $_{N1/2}$-Dry weight $_{N1}$)/ Dry weight $_{N1}$)	(Dry weight $_{N2}$-Dry weight $_{N1}$)/ Dry weight $_{N1}$)
			Mean				
1	13	809,811,813,818,821,822,824,841,846, 847,848,859,863	32.86^{Aa}	1.071^{Cc}	1.069^{Bb}	-0.856^{Cc}	-0.580^{Cc}
2	9	819,826,840,855,857,858,860,867,870	33.80^{Aa}	1.186^{Bb}	1.088^{Bb}	0.659^{Bb}	-0.019^{Bb}
3	8	804,805,806,815,817,837,844,850,	30.35^{Aa}	1.299^{Aa}	1.280^{Aa}	0.701^{Aa}	0.753^{Aa}
Combined	30						

A-C shows very significant differences ($P < 0.01$); a-c shows significant differences ($P < 0.05$).

environment; type 2, the dry weight of the materials growing in $N_{1/2}$ environment increased, but the dry weight in N_2 environment decreased, which had a good adaptability to N-free conditions in the low-nitrogen environment, but a poor adaptability in high-nitrogen environment; type 3, the materials growing in $N_{1/2}$, N_2 environment showed some increase, which had a good adaptability to N-free conditions but did not show obvious post-treatment SPAD regularity; the dry weight of individual plants was more sensitive to N-free conditions than the leaf color. It seemed that rice's response to N-free conditions had a close relationship with its initial nitrogen growth environment.

Physiological analysis of difference in typical typed rice material's response to different nitrogen concentrations

To further compare the physiological basis of the adaptation of different types of rice to the changes of nitrogen concentrations, 6 typical materials were emphatically chosen in this study from the 3 different types of materials in Table 2 to systematically measure their physiological indexes, including R/S ratio, NRA, soluble protein content, and Rubisco contents in different nitrogen concentrations or N-free treatment. As shown in Table 3, some extent of similarity existed in the physiological performance of different types of rice in different nitrogen concentrations and N-free conditions. Individual plant's dry weight reflected the overall performance of the plant's growth and increased in different nitrogen conditions to varying degrees. Compared with N_1 concentration, the R/S ratio did not increase in N_2 concentration, but increased in $N_{1/2}$ concentration; no obvious changes were seen in N-free treatment. Seen from the chlorophyll content, there was a significant increase in N_2 concentration, while no obvious changes in $N_{1/2}$ concentration, and no significant changes in N-free treatment. The soluble protein in the leaf changed in different nitrogen concentrations and N-free treatment; it decreased in $N_{1/2}$ and increased in N_2, and the difference was more significant in N-free treatment. Rice materials did not show obvious changes in Rubisco content in different nitrogen concentrations, but those growing in N_2 saw significant increases after N-free treatment, and those growing in $N_{1/2}$ did not show obvious changes, indicating that they had some response to N-free conditions. Under the treatment of different nitrogen concentrations, the change in the leaf's NRA was similar to that of the soluble protein content: a significant increase in N_2, no obvious increase in $N_{1/2}$, and a decrease in some materials; however, in N-free conditions, there was an obviously induced increase in activities in $N_{1/2}$, but no obvious changes in N_2 or decreases of some materials, which was opposite to changes in Rubisco content. It is possible for materials growing in different nitrogen concentrations, that when those growing in low-nitrogen were exposed to N-free conditions, NRA was induced to increase to more efficiently absorb external nitrogen nutrients, while those growing in high-nitrogen were made up for the

Table 3. Change of R/S ratio, SPAD, soluble protein of leaf, NRA, the content of Rubisco in different types of rice which grow under different environmental N concentration or after N-free treatment.

Number	R/S ratio	Chlorophyll content (mg/g)	Soluble protein of leaf (mg /g FW)	Nitrate reductase activity (μg/gFW.h)	Rubisco content (mg /gFW)	R/S ratio	SPAD	Soluble protein of leaf (mg /g FW)	Nitrate reductase activity (μg/gFW.h)	Rubisco content (mg /gFW)
	After N treatment					**After n-free treatment**				
815(1/2N)	0.381±0.011	6.251±0.24	11.959±0.34	53.350±2.40	6.944±0.19	0.366±0.014	28.3±1.4	7.57±0.31	76.76±2.31	5.39±0.24
815(1N)	0.356±0.012	6.200±0.21	9.970±0.25	41.300±2.14	6.870±0.21	0.356±0.011	30.8±1.2	9.97±0.38	41.3±1.92	6.87±0.21
815(2N)	0.286±0.009	10.506±0.23	18.446±0.54	89.030±4.35	6.564±0.18	0.376±0.011	33.7±1.4	14.52±0.45	28.3±1.23	7.65±0.24
817(1/2N)	0.244±0.008	6.161±0.19	11.544±0.28	40.900±2.49	6.523±0.19	0.400±0.013	31.7±1.2	2.96±0.11	130.85±3.92	6.32±0.33
817(1N)	0.359±0.100	6.100±0.31	10.330±0.25	40.900±2.16	6.890±0.14	0.359±0.011	30.7±1.1	10.33±0.21	40.9±1.85	6.89±0.24
817(2N)	0.304±0.009	8.029±0.31	17.180±0.31	67.170±3.17	6.622±0.16	0.388±0.010	34.4±1.2	19.19±0.87	24.30±1.14	9.10±0.25
840(1/2N)	0.521±0.012	6.732±0.19	12.312±0.27	51.760±2.48	6.200±0.15	0.429±0.012	28.03±1.4	13.25±0.54	80.70±2.94	7.70±0.19
840(1N)	0.364±0.011	6.250±0.24	9.890±0.22	40.510±2.07	6.810±0.17	0.364±0.011	30.2±1.1	9.89±0.23	40.51±1.76	6.81±0.27
840(2N)	0.152±0.007	13.491±0.32	19.463±0.51	91.710±4.65	5.623±0.11	0.355±0.010	30.3±1.3	19.78±0.45	26.96±1.57	11.20±0.57

shortage of external nitrogen supplies by adjusting their internal Rubisco content.

Differences also existed in the adaptability of different types of rice to nitrogen concentration (Table 4). The R/S ratio of 815 and 817 decreased significantly in $N_{1/2}$ and N_2, while the individual plant's dry weight increased. Since their responses to high- and low- nitrogen were reflected in the decrease of the root weight, it seemed that this type had a poor adaptability. The R/S ratio of 840 and 855 also displayed their adaptability to different nitrogen concentrations. That is, the R/S ratio increased in $N_{1/2}$ and decreased in N_2; physiologically, the low-nitrogen condition induced the increase in NRA to absorb external nitrogen nutrients more efficiently, and the materials growing in high-nitrogen conditions utilized the external rich nitrogen sources to the maximum through increasing the synthesis of chlorophyll and soluble protein, thus illustrating a good adaptability to high and low nitrogen conditions. 847 and 848 were morphologically less sensitive to external high and low nitrogen concentrations, showing no obvious changes in the R/S ratio; physiologically, they showed some capacity to increase the synthesis of chlorophyll and soluble protein in N_2, illustrating a medium adaptability to different nitrogen concentrations.

With N-free treatment, materials 847 and 848 which grew in two concentrations both showed a significant increase in the R/S ratio in N-free conditions, but there were no obvious changes in the chlorophyll content. NRA of materials growing in low-nitrogen conditions was induced to increase, and more soluble protein and Rubisco contents were mobilized in materials growing in high-nitrogen conditions to suit the N-free conditions, which illustrated a good adaptability to N-free conditions. Materials 840 and 855 showed similar changes with 847 and 848, but to a smaller degree. Materials 815 and 817 growing in the two concentrations had a dull reaction to N-free condi-

Table 4. Correlation analysis of physiological and growth indexes of nitrogen response in different rice varieties.

Correlation coefficient		Different nitrogen concentration treatment				N-free treatment				
		Soluble protein	Rubisco content	Chlorophyll content	Nitrate reductase activity	R/S ratio	SPAD	Soluble protein of leaf	Rubisco content	Nitrate reductase activity
Different nitrogen concentration treatment	R/S ratio	-0.605**	0.064	-0.0104	-0.317	0.513*	-0.150	-0.099	-0.195	0.372
	Soluble protein		0.051	-0.133	0.915**	-0.017	0.113	0.447	0.534*	-0.416
	Rubisco content			-0.056	0.209	0.562*	0.493*	0.746**	0.353	-0.106
	Chlorophyll content				-0.181	-0.210	-0.074	-0.103	-0.137	-0.127
	Nitrate reductase activity					0.259	0.226	0.589*	0.556*	-0.357
N-free treatment	R/S ratio						0.459	0.437	0.341	0.346
	SPAD							0.572*	0.473*	-0.295
	Soluble protein								0.719**	-0.537*
	Rubisco content									-0.423

tions, showing no obvious changes in the R/S ratio. Despite the nitrate reductase of the materials growing in $N_{1/2}$ showed a strong inductive capability in N-free conditions, it was not enough to offset the influence on the materials by N-free conditions, which was shown by the significant decrease in soluble protein and Rubisco contents. In addition, the nitrate reductase was not induced to increase in N-free situation for materials growing in N_2, thus, it was clear that this type of materials had a poor adaptability to N-free conditions.

Correlation analysis of the physiological indexes of rice's response to different nitrogen concentrations

The correlation analysis was conducted for various physiological indexes of rice in different levels of nitrogen (Table 4), in which the R/S ratio in different concentrations of nitrogen was significantly or highly significantly correlated with the soluble protein in different concentrations of nitrogen and the R/S ratio in N-free treatment, with the correlation coefficients of -0.605** and 0.513** at the level of 0.01, thus, clearly indicating that, the growth of rice in different concentrations of nitrogenhad a close relationship with the synthesis of its protein and its growth in N-free conditions. The change in soluble protein in different concentrations of nitrogen had a significant and highly significant correlation with NRA in different concentrations of nitrogen and the Rubisco content in N-free treatment, with the correlation coefficients of 0.915** and 0.534* at the level of 0.05 and 0.01, respectively, clearly showing that the leaf's soluble protein was the important material basis for rice's response to different concentrations of nitrogen. It seemed that the R/S ratio can also be used as a simple and efficient screening index for rice's response to the change in nitrogen concentration in the field cultivation.

DISCUSSION

Nitrogen is one of the essential nutrient elements to plant and it is known that multiple mechanisms are employed by the plant to adapt to the changes of nitrogen environment (Zhang and Forde, 1998; Ladha et al., 1998). The root system is the first part in the plant that feels and absorbs nitrogen and it is very sensitive to nitrogen distribution and availability in the soil. Nitrogen supply is the direct external signal or indirectly affects the nitrogen distribution in the plant to influence the growth of the root system. The nitrogen in the form of nitric nitrogen and ammonium nitrogen is necessary for plant growth and it can adjust the growth of the root system (Tian and Mi, 2008).

Different rice varieties show desperate responses to nitrogen, thus, leading to significant difference in nitrogen's utilization ratio (Datta and Broadbent, 1990; Wu and Tao, 1995; Inthapanya and Sipaseuth, 2000; Piao et al., 2001; Koutroubas and Ntanos, 2003; Piao et al., 2003; Samonte et al., 2006; Shrestha et al., 2010). Nitrogen application rates also have influence on the production and output of rice's dry matter, which have been widely reported. (Liu et al.,2009;Hu et al.,2009) However, these studies were mostly focused on typical nitrogen in different rice varieties. Although, there are a few reports on hybrid combination, they are usually concerned with the morphological and physiological characteristics changes in the aerial parts and few are on the nitrogen screening indexes about both the aerial and underground parts. The nitrogen treatment in those studies is again limited to high and low nitrogen treatment and those concerned rice's adaptability to the change of nitrogen in natural environment are seldom seen. In this study, rice's adaptability to nitrogen was divided into 6 types based on the adaptability of 70 hybrid combinations in selection breeding to different concentrations of nitrogen and through the R/S ratio screening. And the growth materials' adaptability to N-free conditions in different nitrogen conditions was divided into 3 types through the further combined screening of the common nitrogen adaptability screening indexes, such as the dry matter weight and SPAD value and the varieties' physiological basis was compared by 6 typical materials' R/S ratio, chlorophyll content, soluble protein content, Rubisco content and NRA. The results showed that, the types of rice with good adaptability to different concentrations of nitrogen had certain characters in common: morphologically, the root weight increased in low nitrogen conditions and the leaf and stem weight increased in high nitrogen conditions; physiologically, NRA was induced to increase low nitrogen conditions to absorb external nitrogen nutrients more efficiently and the materials in high-nitrogen environment could maximally utilize the external rich nitrogen sources by increasing the synthesis of chlorophyll and soluble protein. Special attention should be paid to the result that the response of hybrid rice materials to N-free conditions was that when the materials in low-nitrogen environment encountered N-free conditions, nitrate reductase activity was induced to increase to absorb external nitrogen nutrients more efficiently, while the materials in high-nitrogen environment made up for the shortage of external nitrogen supplies through the mobilization of internal Rubisco content. It was thus clear that, the rice variety with a good adaptability to nitrogen made up for the shortage of external nitrogen supply by flexibly regulating the parts growing above and under the ground, increasing NRA induced by low-nitrogen, synthesizing more chlorophyll and soluble protein in high-nitrogen, inducing NRA and mobilizing Rubisco content in N-free situations. Nitrate reductase might be the first process for rice to adapt to the nitrogen concentration, which increases the absorption of nitrogen through increasing sources, but its adjustment capability is limited. Rubisco content is the second process when nitrate reductase fails by reducing consumption: Rubisco content is mobilized to compensate for the shortage of external nitrogen supplies, thus, increasing its internal adaptability to the poor nitrogen environment. The results of this study are a good supplement to the research in hybrid rice's responses to the change in nitrogen.

The genetic control of the nutrient efficiency of a large amount of nutrient elements is rather complicated, which is manifested in continuous variation and has the feature of quantitative genetic trait (Zhang and Zhang, 1994). Nitrogen's absorption and utilization in rice is greatly influenced by the environment and also other nutrient elements such as P and K. With molecular marker technology, there have been studies on the quantitative trait locus analysis about plant's nitrogen utilization efficiency in recent years (Yamaya et al., 2002), but there is still a long way to go to discuss the genetic law of rice's nitrogen utilization trait and performance from the genetic level. Therefore, the evaluation indexes for nitrogen efficiency applicable in production and a set of simple screening system for rice germplasm resource would be helpful to further research into the physiological and biochemical characteristics and molecular ecological mechanisms of high nitrogen efficient nutrients for crops, which might be one of the important factors that contribute to the breakthrough in the efficiency research of crop nitrogen fertilizer in the future. At present, the diagnosis indexes frequently used in production for the nitrogen nutrient for the aerial part contain the plant's total nitrogen content, chlorophyll content of the nitrogenous leaves, the nitrogen content of tissue juice, and SPAD, etc (Peng et al., 1993); Zeng et al. (2007) also proposed that, GS activity and soluble protein content had important reference value for evaluating rice's nitrogen utilization efficiency (Zeng et al., 2007). The results of this study show that, the R/S ratio can also be used as a simple index for the diagnosis of nitrogen nutrient in rice, which is a helpful supplement to include the root’s response to nitrogen. In short, the absorption and utilization of nitrogen in rice is a dynamic process: in addition to the differences of various rice varieties and genes related to nitrogen's utilization, the determination of various indexes of its growth and development has an important role in judging nitrogen utilization efficiency in rice (Jiang and Cao, 2002). Nitrogen loss may be one of the major causes that inhibit photosynthesis, chlorophyll synthesis, relevant genes of plasmid protein synthesis and secondary metabolic responses (Scheible and Morcuende, 2004). The development of genomics, bioinformatics and systematology is revealing the regulatory mechanism of plant responses to different levels of acquired nitrogen including transcription and post-transcriptional regulation. This study included research clues on the physiological mechanism of different rice materials' response to different concentrations of nitrogen and that of the materials growing in different nitrogen concentrations and later exposed to N-free conditions, which will provide reference for relevant researches on the molecular mechanism ofrice's response to nitrogen and the reasonable fertilization.

Conclusions

In this paper, physiologically, the rice materials with good adaption of N were due to higher nitrate reductase activity (NRA) at low nitrogen conditions, more chlorophyll content and soluble protein in high nitrogen conditions and the Rubisco mobilized in N-free conditions as well.

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