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Drought-Resistance Index in Rice Backcross Lines after Anthesis

Wu Na, Guan Yongsheng and Shi Yan
Dryland Technology Key Laboratory of Shandong Province in Laiyang Agricultural University, Qingdao, 266109, China

Abstract: In this study, many indexes correlated with drought resistance including yield components, chlorophyll content, the content of proline, the content of malondiadehyde (MDA), the activity of superoxide dismatase (SOD), the content of peroxides (POD) and catalase (CAT) activity soluble protein content and leaf area in flag leaves were measured under water stress after flowering in five rice backcross combinations. The indexes for drought resistance were screened by the correlation and the gray relationship analysis, under water stress after flowering. The results indicated that after flowering, rate of seeds fertilization was significantly correlated with yield remarkably. And proline content, MDA content in flag leaf and leaf areas were significantly influenced with drought resistance in rice, which indicated that it is feasible to predict the drought resistance in rice after flowering. According to the gray relationship analysis, ability of drought resistance in five rice backcross combinations is the following, the combination 5> combination 1>combination 4>combination 3>combination 2. Results were generally consistent with the performance in field, which indicated that it is reasonable to predict the drought resistance in rice after flowering with the four indexes screened out in this study.

Key words: Rice backcross lines, drought resistance, identification index

INTRODUCTION

With the increasing deficit of global water resource and increasingly seriousness of drought, water shortage is crucial for restricting agricultural development in China. Breeding rice cultivars with drought resistance and cultivating them in the dryland are helpful not only to save water resource and to steady yield, but also to increase yield and save energy and to decrease environmental pollution (Cheng, 2005). In China, rice is the top first crop in area planted to rice and the total yield, which approximately accounts for 28% of total crop area, 40% of the total yield (Wang and Zhau, 2000). Furthermore, rice is one of the main irrigation crops with large water usage, around 65% agricultural water consumption. Drought has occurred more and more frequently in the past few years. In china, the arid and half arid areas approximately account for 51% of the cultivated ones, mainly distributed to Northwest and Northern China. In Southern China, although the rainfall is abundant, distribution is uneven each month, which cause the drought to happen with strong seasonal characteristic (The People's Republic of China state Statistical Bureau, 2003; Shan, 2004; Wang, 2000; Shao-Zhong, 1998). Drought has been one of the most limited factors for rice production. Therefore, studies on drought resistance in rice have become increasingly important proposals for rice scientific researches. Regarding the more complex mechanism of drought resistance in rice, a series of morphological, developmental, physiological and biochemical identification methods and indexes related to drought resistance in rice were adopted by international scientists and some indexes were located genes with molecular marker.

Leaf water potential is considered to be a reliable parameter for quantifying plant water stress response. Singh *et al*. (1990) observed significant differences in water potential among wheat genotypes under drought stress. Sinclair and Ludlow (1985) proposed that leaf Relative Water Content (RWC) was a better indicator of water status than was water potential. Canopy temperature is also related to water stress. Ehler *et al.* (1978) reported that the canopy temperature provided a good indication of the plant water potential of wheat when comparing environments with varying degrees of water stress. This study investigated the water relations of wheat under different levels of drought stress. Some osmotic adjustment matters in plant cells, such as SOD, POD and MDA and so on have been known as the important pathway for drought tolerance. However, the application values of most indexes were doubted because of the indistinct relationships between them and yield (Cheng, 2005).

Corresponding Author: Shi Yan, Dryland Technology Key Laboratory of Shandong Province in Laiyang Agricultural University, Qingdao, 266109, China

Statistics indicated that the reduction of yield caused by drought was higher than other factors (Wang and Jun, 2000). Furthermore, drought could also affect the quality and the area planted in rice. Therefore, the reasonable evaluation, identification and screening for drought tolerance in rice, will be benefit not only to the production increase and stability, but also to saving the agricultural water. The aim of this study to screen the index for drought tolerance, as the theory basis of breeding and cultivation for drought resistance in rice.

MATERIALS AND METHODS

Experimental design and treatments: This study was conducted in 2005-2006 in Laiyang Agriculture University in China, which lied in E 120.7°, N36.9°, warm temperate zone, half moist monsoon climate, the annual mean temperature 11.2°C, frost-free period 209 ~ 243 days, the annual rainfall is 779. One millimeter, mostly concentrates in July, August and September. The soil type is the loam; the fertility level is high and the content of basal soil nutrients of experiment were following, 1.24% organic fertilizer, 1.04 mg kg^{-1} total nitrogen, 86.54 mg kg^{-1} available nitrogen, 24.58 mg kg^{-1} available phosphorus, 85.72 mg kg^{-1} available potassium. Seeds was sowed on May 10, 2 lines/Strain, 12 holes/Line, 2 grains/Hole, thickness of sowing 20×10 cm^2. The experiment set up two treatments, the control treatment and the water stress treatment, 3 replications. Water stress treatment maintains 10 cm water on the field, before anthesis the control treatment keeps the same with the water stress treatment, after anthesis it was stopped supplying water.

In this study, the following 5 combinations were used; in combination 1, C418 and zaoxianl4 as the parents, by the C418/zaoxian 14//C418///C418 way carries on the backcross, altogether screens out 6 backcrosses descendants, DLR34-DLR39; Combination 2 takes C418 and yuexiangzhan as the parents, by the C418/yuexiangzhan//C418///C418 way carries on the backcross, altogether screens out 66 backcrosses descendants, DLR55-DLR61, DLR63-DLR82, DLR84-DLR103, DLR105-DLR123; Combination 3 takes C418 and Manawthukha as the parents, by the C418///C418//Manawthukha/C418 way carries on the backcross, altogether screens out 19 backcrosses descendants, DLR2-DLR20; Combination 4 takes C418 and C71 as the parents, by the C418///C418//C71/C418 way carries on the backcross, altogether screens out 10 backcrosses descendants, DLR23-DLR32; Combination 5 takes C418 and Teqing as the parents, by the Teqing/C418//C418///C418 way carries on the backcross, altogether screens out 12 backcrosses descendants, DLR42-DLR53 (Table 1).

The Anhui Province Academy of Agricultural Sciences Rice Research Institute and the Chinese Academy of Agricultural Sciences Plasma Physics Research Institute use the ion injection method radiating the varieties of jiaxian293 and Zhennong 10 to select and get the variety zaoxianl4, In August, 1999. It passed the examination and approves through the Anhui Province crops examination and approval committee. C418, was a kind of Japanica, with more indica genetic morphological character and comprehensive appetency, from the hybridization of wanlun 422/miyang 23(indica) in Liaoning Academy of Agricultural Sciences in 1980's. The Guangdong Province Academy of Agricultural Sciences Rice Research Institute use 32 Ai/Qingxiangzhan for the female parent, the zhongyou/Guangxi fragrant rice for the male parent, after the compound hybrid, the zaoxian and center ripe general high quality variety Yuexiangzhan was bred in 1999.

Measurements: The physiological and biochemical indexes were measured in rice sword leaves.

Membrane fat peroxidation product MDA was measured with the pair of group spectrophotometer method (Tang, 1999), free proline content, with acidic hydration ninhydrin method (He, 1993), Soluble protein content with coomassie blue method (Zhang, 1992), POD with guajacolum method (Amalo, 1994), SOD, with the method of Gisnnopolitis and Ai-guo Wang, the CAT with potassium permanganate titrim-etric method (Wang *et al*., 1983), green leaf area with the AM100 leaf area meter, chlorophyll content using MINLTA5SPAD-502CHLOROPHYLLMETER to measure the function leaf in field at 9: 30-11: 30 in the morning, separately and the tiller numbers, the total dry weight and the ratio of root to shoot ratio were measured according to indexes measurement.

Data and statistical analysis: The results from indexes were analyzed with the correlation and the gray relationship in control and water stress treatments, so we could find the most related factors with drought, including the physiological and morphological traits and evaluated comprehensively the ability of drought tolerance in rice with (1) RER (Relative Effective Rate) (%) = the difference between the value from control treatment and the value from water stress treatment/the value of control treatment ×100, was used to compare the ability of drought resistance between the different combinations. (2) The practical ability of drought resistance in each combination was comprehensively evaluated by the gray relationship analysis with the following formula:

Table 1: The effect under water stress after flowering on drought-resistance coefficient in five rice combinations

Combinations	Cultivars	Drought resistance coefficient	Cultivars	Drought resistance coefficient	Cultivars	Drought resistance coefficient	Cultivars	Drought resistance coefficient
Combination 1	C418	0.74	DLR34	0.87	DLR36	0.94	DLR38	0.91
	zaoxian14	0.76	DLR35	0.85	DLR37	0.90	DLR39	0.88
Combination 2	C418	0.74	DLR71	0.84	DLR89	0.84	DLR107	0.66
	yuexiangzhan	0.75	DLR72	0.73	DLR90	0.73	DLR108	0.85
	DLR55	0.74	DLR73	0.74	DLR91	0.91	DLR109	0.93
	DLR56	0.74	DLR74	0.92	DLR92	0.92	DLR110	0.82
	DLR57	0.64	DLR75	0.61	DLR93	0.71	DLR111	0.93
	DLR58	0.74	DLR76	0.49	DLR94	0.74	DLR112	0.92
	DLR59	0.79	DLR77	0.81	DLR95	0.86	DLR113	0.92
	DLR60	0.74	DLR78	0.67	DLR96	0.70	DLR114	0.74
	DLR61	0.84	DLR79	0.83	DLR97	0.72	DLR115	0.67
	DLR63	0.83	DLR80	0.63	DLR98	0.92	DLR116	0.71
	DLR64	0.87	DLR81	0.87	DLR99	0.91	DLR117	0.72
	DLR65	0.91	DLR82	0.88	DLR100	0.90	DLR118	0.87
	DLR66	0.78	DLR84	0.67	DLR101	0.87	DLR119	0.46
	DLR67	0.84	DLR85	0.54	DLR102	0.90	DLR120	0.92
	DLR68	0.79	DLR86	0.92	DLR103	0.90	DLR121	0.70
	DLR69	0.90	DLR87	0.90	DLR105	0.87	DLR122	0.74
	DLR70	0.92	DLR88	0.74	DLR106	0.92	DLR123	0.47
Combination 3	C418	0.74	DLR6	0.93	DLR12	0.93	DLR18	0.89
	Manawthukha	0.82	DLR7	0.80	DLR13	0.92	DLR19	0.84
	DLR2	0.90	DLR8	0.91	DLR14	0.91	DLR20	0.87
	DLR3	0.88	DLR9	0.92	DLR15	0.90		
	DLR4	0.80	DLR10	0.89	DLR16	0.94		
	DLR5	0.90	DLR11	0.80	DLR17	0.65		
Combination 4	C418	0.74	DLR24	0.92	DLR27	0.81	DLR30	0.89
	C71	0.78	DLR25	0.90	DLR28	0.87	DLR31	0.88
	DLR23	0.92	DLR26	0.82	DLR29	0.82	DLR32	0.86
Combination 5	C418	0.74	DLR44	0.98	DLR48	0.93	DLR52	0.92
	Teqing	0.85	DLR45	0.95	DLR49	0.93	DLR53	0.94
	DLR42	0.90	DLR46	0.93	DLR50	0.98		
	DLR43	0.94	DLR47	0.90	DLR51	0.99		

Drought resistant coefficient is in the yield level, namely Drought resistant coefficient = water stress yield/control yield, the same as below

$$\gamma_i = \frac{1}{n}\sum_{k=1}^{n}\varepsilon_i(k)$$

n, indicated the number of varieties; k, the number of traits; ε, correlation coefficient; I, the order of varieties (Meng, 2003).

RESULTS

The effect on drought-resistance coefficient in five combinations under water stress after flowering: The coefficient above 0.85 in combination 1, showed descendants had strong drought tolerance. The coefficient above 0.85, accounted for 40.91% of descendants in combination 2; below 0.85, for 60.09%; between 0.70 and 0.85, for 40.91%, with large proportion, which indicated the descendants in this combination had low ability of drought resistance. Meanwhile the genes from two parents (C418 and yuexiangzhan) could be pyramided through the backcross, but the gene for drought resistance (Table 1) could not be completely pyramided or had the poor stability. Drought-resistance coefficient, above 0.85, accounted for 73.68% in combination 3; 80%, above 0.85 in combination 4; all above 0.90 in combination 5, which showed the descendants in this combination resisted the drought strongly. Furthermore, the backcross could pyramid the genes for drought tolerance from two parents (C418 and yuexiangzhan). And the genes for drought resistance could fully express in the backcross descendants. The effect of water stress after flowering on the drought-resistance coefficient in these five rice combinations were following: combination 5 > combination 1 > combination 4 > combination 3 > combination 2.

The correlation coefficient between yield components in different rice combinations: In Table 2, the correlation coefficients between each component and yield were as follows: The rate of seeds fertilized > filled grains number > 1000-grain weight > total florets number > effective tiller > panicle length. The rate of seeds fertilized, filled grains number, 1000-grain weight and total florets number were significantly related to yield, with correlation coefficient 0.928, 0.917, 0.898 and 0.847, respectively; The effective tiller number remarkably negatively correlated with yield, filled grains number and the rate of seeds fertilized; the correlation between panicle length and 1000-grain weight reached remarkable level, without significant correlation with the other factors.

The correlation analysis between physical and physiological indexes and yield in different rice combinations: Table 3 showed: the free proline content and green leaf area were significantly positively correlated with yield, with correlation coefficient 0.618 and 0.591, respectively; MDA content was remarkably negatively related to the yield, with the correlation coefficient-0.608; The above ground dry weight, total dry weight, chlorophyll content were significantly positively correlated with yield, with the correlation coefficient 0.537, 0.506 and 0.501, respectively. The correlation coefficient between Indexes and yield can be seen as follows: free proline content > MDA content > green leaf area > aboveground dry weight > total dry weight > chlorophyll content > SOD activity > POD activity > soluble protein content > tiller number > root/shoot ratio > CAT activity. Meanwhile, the significant correlations among indexes, except for yield and tiller number, could be seen.

The gray relationship analysis between different backcross combinations: Drought resistance is a complex character. It is more realistic to apply many indexes to comprehensive analyze and evaluate. The gray relationship analysis, as a comprehensive analysis method, may combine many indexes as one index-relevancy, which can be used to evaluate the drought resistance in different rice backcross combinations (Meng, 2003). The optimum values in every average RER

Table 2: The correlation coefficient between of yield and its components in different rice combinations

Parameters	Yield	Effective tiller	Panicle length	Total florets No.	Filled grains No.	Seed setting rate	1000-grain weight
Yield	1	-0.427*	0.284	0.847**	0.917**	0.928**	0.898**
Effective tiller		1	0.129	-0.321	-0.449*	-0.439*	-0.221
Panicle length			1	0.419	0.220	-0.171	0.590**
Total florets No.				1	0.897**	0.391	0.955**
Filled grains No.					1	0.751**	0.859**
Seed setting rate						1	0.369
1000-grain weight							1

*, ** Indicated significant correlation at 0.05 and 0.01 threshold

Table 3: The correlation coefficient among RER (%) of yield and 12 indices

Parameters	Yield	Tillers	Leaf area	Root-shoot ratio	Gross dry weight	Dry weight above ground	Chlorophyll content	MDA	Soluble proline	Protein	SOD	POD	CAT
Yield	1	0.306	0.591**	0.149	0.506*	0.537*	0.501*	-0.608	0.618**	0.312	0.395	0.321	0.120
Tillers		1	0.009	0.157	0.416	0.438*	0.529*	-0.289	0.726**	0.337	0.489*	0.379	0.221
Leaf area			1	0.463*	0.853**	0.848**	0.550**	0.719**	0.387	0.208	0.577**	0.692**	0.467*
Root-shoot ratio				1	0.709**	0.656**	0.626**	-0.788**	0.585**	0.886**	0.827**	0.794**	0.957**
Gross dry weight					1	0.987**	0.796**	-0.872**	0.735**	0.567**	0.877**	0.916**	0.708**
Dry weight above ground						1	0.778**	0.843**	0.727**	0.516	0.857**	0.895**	0.652**
Chlorophyll content							1	0.925**	0.928**	0.716**	0.908**	0.855**	0.662**
MDA								1	-0.816**	-0.748**	-0.911**	-0.893**	-0.825**
Proline									1	0.679**	0.853**	0.751**	0.631**
Soluble protein										1	0.836**	0.771**	0.889**
SOD											1	0.963**	0.805**
POD												1	0.768**
CAT													1

*, **indicated significant correlation at 0.05 and 0.01 threshold

Table 4: The average RER(%) of important characters among different rice combinations

	RER average									
Rice combination	Proline	Leaf area	Soluble protein	SOD	POD	CAT	MDA	Spikelets weight per plant	Root-shoot ratio	Root dry weight	Dry weight above ground
Optimal value	-72.5	3.2	10.8	-21.7	-35.6	-45.6	17.3	-16.3	-128.9	-50.7	-10.6
Combination 1	-31.8	9.8	10.8	-18.9	-33.1	-37.9	17.3	7.4	-128.9	-50.7	-0.9
Combination 2	13.5	18.7	35.9	-3.1	-10.7	-22.7	-8.8	-4.9	-44.9	24.3	-4.4
Combination 3	16.1	12.9	26.8	-6.4	-15.3	-28.0	-3.5	7.4	-49.8	29.1	-7.5
Combination 4	-9.8	15.2	27.9	-12.8	-27.8	-31.1	-0.9	13.1	-78.9	-11.9	-3.3
Combination 5	-72.5	3.2	12.4	-21.7	-35.6	-45.6	15.8	-16.3	-90.8	-32.1	-0.8

Table 5: The gray relevancy and ranks among different rice combinations

	Combination 1	Combination 2	Combination 3	Combination 4	Combination 5
Relevancy	0.8669	0.6999	0.7689	0.8001	0.9282
Sequencing	2	5	4	3	1

of indexes in all the five combinations, were used as a combination. So each index is of equally importance, namely equal weight.

The analysis results were showed in Table 4. The bigger the relevancy is, the stronger the drought resistance is. As was shown in Table 5, the combination 5 had the strongest drought resistance (r_i = 0.9282), combination 1 ranked the second (r_i = 0.8669) and combination 2 was the weakest (r_i = 0.6999). The above results were consistent with the performance in the field.

DISCUSSION

The drought resistance in plants is one complex quantity genetic feature affected by many factors (Zhang, 2005). The drought resistance of a variety in the specific area was determined by its own physiological resistance, the structure characteristic as well as the growth and development rhythm combined with the agriculture climatic factor (Jin, 1999). The crops drought resistance is not only influenced by crops type, variety, genotype, physical characters and physiological biochemical response, but also the drought degree, drought period and the drought duration, it is the result of interaction between waters and the physiological function in plants, meanwhile it is also the result of interaction between plants and environment (Luo, 2001). The different variety has differently drought resistance mechanism, for a variety, which is also different, at the different stages. So some single mechanism can not evaluate the drought resistance effectively and accurately (Gong, 1989). Therefore, it is effective to select several major ones from many physiological and biochemical indexes which remarkably make effect on the drought resistance.

In this study, the five backcross combinations were used and all the yield components, the leaf area, the chlorophyll content, the content of proline and SOD and POD and CAT and MDA and soluble protein of flag leaf were measured under the water stress condition after flowering. The Pearson correlation index was conducted. The results showed that the rate of fertility grains was significantly positively correlated to the yield and the content of proline and MDA and leaf area etc, significantly affected the drought tolerance in rice, so the above traits can be thought as the traits for identification. According to the Pearson correlation, the ability to the drought tolerance between the five combinations ranked as follows, the combination 5> combination 1> combination 4> combination 3> combination 2 and the results were consistent to the field. And the RER of every index indicated the drought tolerance of plants, eliminated genetic difference between varieties, so that drought resistance was exactly reflected (Wang and Jun, 2005). Meanwhile, the compare can be conducted, not only between the same indexes but also between the different indexes, with the relative value. The change tendency among indexes and credibility can be seen obviously (Wang and Jun, 2005).

In view of the damage brought with drought resistant, some physiological and physical indexes have already been studied and the different identification indexes for drought resistant have been put forward. The comprehensive indexes have been accepted to identify the drought resistant in plants and have also been reported in many articles. This indicated that it is practicable to identify the drought tolerance with those traits and analyze the crop drought tolerance with gray relations methodology.

However, the complex drought resistance occurs at any growth stage in crop, with the different response and the different mechanism to drought tolerance, in this study, these traits measured need to confirm if it is suitable to apply these indexes to other growth stages. It is suggested that we should explore complex drought resistance identify indexes and methods of rice positively in the future (HR *et al*., 2004, 2006) and combine the morphology, physiological, biochemical and growth indexes as well as scientific quantity analysis method to provide a scientific systematical and perfect system for rice drought resistance identity and to provide theory instruction for rice drought resistance breeding and cultivation.

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