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Breeding of New Upland Rice Variety 'Zhongkexilu 2' by Molecular Marker-assisted Selection

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Abstract In order to cultivate new upland rice varieties with high yield and wide adaptability, which is suitable for Yunnan, Hainan and other tropical and subtropical areas, a new temperature sensitive upland rice variety 'Zhongkexilu 2' was cultivated by hybridization and backcrossing between rice variety 'Minhui 63' and upland rice variety 'Lu 46'. The upland adaptive QTLs were introduced through the molecular marker assisted selection technology and pedigree method. The variety participated in evaluation trial at 3 sites in two years in Hainan. The average yield of multi-point regional trial in two years were 4 914 kg/hm^2, which increased by more than 25.5% compared with the contrast variety 'Yunlu 201'. This shows that it has a strong potential for increasing production. The plant height was 105 cm, with panicle length of 22 cm. Effective panicles number per plant were 7, and grain number per panicle was 144. Seed setting rate was 89.2%, and the thousand grain weight was 24.1 g, showing the moderate plant type and panicle traits. The drought resistance of 'Zhongkexilu 2' was identified. The result showed that its drought resistance was basically the same as that of the upland parent 'Luyin 46', and it was significantly stronger than that of the parent 'Minghui 63'. It was approved by Hainan Variety Certification Committee in 2019 (Qiongshen Rice 2018033). New varieties with high yield and drought resistant were cultivated through the molecular marker assisted selection technology and pedigree method, which was of great significance to promote the regeneration of upland rice varieties and the development of upland rice industry.
Keywords 'Zhongkexilu 2'; Upland rice; Molecular marker-assisted selection; Pedigree method

China is a country with frequent droughts. Perennial droughts in Northwest and North China and seasonal droughts such as summer droughts and autumn droughts in South China pose a serious threat to China's rice production (Luo et al., 2000; Luo, 2018). There is a large-scale drought in the central and western regions of Hainan province all year round. It is estimated that the heavy drought area is about 88.27 hm^2, accounting for about 30% of the total area of Hainan Province, which seriously restricts rice production. Breeding new varieties of high-yield and stress resistant upland rice is one of the important measures to deal with the drought.

Dry rice, also known as upland rice, has strong drought resistance and grows on mountain slopes. It has a long planting history in Southwest and South China. In recent years, it has attracted more and more attention of breeders. Since the 1990s, due to China's gradual attention to the water resources crisis, the research and breeding of upland rice have developed rapidly. In 2000, the Ministry of Agriculture of the PRC organized a national regional trial of upland rice, which put China's upland rice breeding on the right track. In recent years, China National Rice Research Institute and China Agricultural University and other scientific research institutes have jointly established a "Cooperative Group for Tackling Key Problems of Upland Rice", which has made good progress in the breeding of new varieties of upland rice. Yunnan Province has explored and innovated a batch of new upland rice materials with Yunnan characteristics, becoming the province with the largest upland rice resources at home and abroad in China. It has formed its own advantages and characteristics in the field of research and utilization of upland rice, and is at the forefront of the country. The new upland rice varieties represented by 'IRAT104', 'Luyin 46', 'Yunlu 103' and 'Yunlu 142' have played an important role in the upland rice production of Yunnan and surrounding countries. Shanghai Agrobiological Gene Center is committed to the breeding of water-saving and drought resistant rice varieties, and has bred Hybrid Rice Varieties 'Hanyou 2', 'Hanyou 3' and 'Hanyou 113', which have been widely popularized in northern China (Mei et al., 2007; Yu et al., 2010, Molecular Plant Breeding, 8(6): 1177-1179; Liu et al., 2013; 2016). The Food Crop Research Institute of Hainan Academy of Agricultural Sciences bred the upland rice variety 'Shanlanlu 1' in 2017, which is significantly higher than the local variety, making Hainan 'Shanlan' rice have the yield conditions for industrial and commercial development (Cai et al., 2019). In tropical and subtropical areas such as Hainan and Yunnan, the overall yield of the promoted upland rice varieties is not high, and the promotion area is small. However, the upland rice promoted in Hainan is mainly 'Shanlan' rice, most of which are photosensitive and the yield is not high. Therefore, breeding new upland rice varieties with high yield, wide adaptability and stress resistance through molecular marker-assisted selection breeding is a direct and effective way to improve the yield of upland rice, which will provide a basis for variety renewal and diversified application.

By summarizing the breeding process, variety performance and cultivation techniques of 'Zhongkexilu 2', this study aims to provide a theoretical basis for upland rice production in Hainan, to breeding a new variety of upland rice with high yield, wide adaptability and stress resistance that is suitable for planting on dry slopes in Hainan and Yunnan, and to master its characteristics and utilization value.

1 Results and Analysis

1.1 Yield data of 'Zhongkexilu 2'

For two consecutive years, the experiment had been conducted in the east, west, north and south of Hainan to determine the yield (Table 1). The results showed that the average yield of the three test sites in Hainan Province in 2016 was 5 184 kg/hm^2, which increased by 26.93% compared with the control variety 'Yunlu 201'; In 2017, the average yield of the three test sites was 4 645 kg/hm^2, which increased by 23.90% compared with the control variety 'Yunlu 201'. The comprehensive analysis of the two-year experiment showed that the average yield of 'Zhongkexilu 2' was 4 914 kg/hm^2, which was 25.49% higher than that of the control variety 'Yunlu 201'. On the whole, 'Zhongkexilu 2' showed an increased yield at all test sites, with most of the increase reaching more than 20%, indicating that the variety has a significant increase in yield and has a strong potential for popularization and application in all cities and counties of Hainan.

Table 1 Yield performance of 'Zhongkexilu 2' in each demonstration site

Time	Site	Longitude and latitude	Area (hm²)	'Zhongkexilu 2' Yield (kg)	'Yunlu201' Yield (kg)	Increased yield (%)
The second crop of 2016	Baoyou town of Ledong county	18°45'N, 109°11'24"E	1.87	5 169	4 264	21.2**
The second crop of 2016	Sigeng town of Dongfang county	19°13'48"N, 108°41'24"E	1.60	4 942	3 849	28.4**
The second crop of 2016	Yongfa town of Chengmai county	19°45'N, 110°12'E	0.27	5 442	4 139	31.6**
Average of 2016	-	-	-	5 184	4 084	26.9**
The second crop of 2017	Baoyou town of Ledong county	18°45'N, 109°11'24"E	2.47	5 468	4 264	27.5**
The second crop of 2017	Yelin town of Lingshui county	18°31'48"N, 110°3'E	0.53	4 449	3 800	17.1**
The second crop of 2017	Lingkou town of Dingan county	19°21'N, 110°18'36"E	0.40	4 018	3 183	19.3**
Average of 2017	-	-	-	4 645	3 749	23.9**
Average of 2 years	-	-	-	4 914	3 916	25.5**

Note: **indicate most significant difference (p<0.01)

1.2 The main agronomic traits of 'Zhongkexilu 2'

In order to fully understand the traits of 'Zhongkexilu 2', 50 plants were randomly selected in the field to investigate their main agronomic traits and take the average value (Table 2). It can be seen that 'Zhongkexilu 2' has moderate plant height, many grains per panicle and high seed rate (Table 2). In addition, the whole growth period is 105~130 days, with moderate plant type, stout stems, good lodging resistance, strong tillering ability and long grain shape; The leaf color is dark green, the glume tip is purple, the glume shell is yellowish brown, the color turns well in the later stage, the green branches are waxy, and there is no premature aging.

Table 2 Results of the main agronomic traits of 'Zhongkexilu 2'

Zhongkexilu 2	Plant height (cm)	Panicle length (cm)	Effective panicle number per plant	Grain number per panicle	Seed rate (%)	1000-grainweight (g)
Group 1	105	21.3	7	136	88.5	22.9
Group 2	105	22.0	7	153	87.8	25.2
Group 3	105	23.7	8	143	91.4	24.3
Mean value	105	22.3	7	144	89.2	24.1

1.3 Indexes of rice quality

The indexes of rice quality were measured according to the standard of Cooking Rice Variety Quality (NY/T593-2013) of the Ministry of Agriculture of the PRC (Table 3). By comparing the standard of Cooking Rice Variety Quality (NY/T593-2013) of the Ministry of Agriculture of the PRC, the results showed that the brown rice rate of 'Zhongkexilu 2' reached 81.8% and the gel consistency reached 69 mm, both of which reached the standard of grade I; The aikali spreading value was grade 5.0, reaching the standard of grade III; The grain length was 5.8 mm and the length-width ratio was 2.4, which belonged to medium grain type. The other indexes such as chalkiness degree and pellucidity were general. It showed that the rice quality of 'Zhongkexilu 2' was above medium.

Table 3 Rice quality analysis of 'Zhongkexilu 2'

Index	Standard	Result	Single decision (Grade)
Brown rice rate (%)	⩾77.0	81.8	I
Milled rice rate (%)	-	67.1	-
Grain length (mm)	Long grain shape: >6.5 Medium grain shape: 5.6-6.5 Short grain shape: <5.6	5.8	Medium grain
Length-width ratio	-	2.4	-
Chalkiness degree (%)	⩽5.0	6.4	General
Chalkiness rate (%)	-	41	-
Pellucidity (Grade)	⩽2	3	General
Aikali spreading value (Grade)	⩾5.0	5.0	III
Gel consistency (mm)	⩾50	69	I
Amylose content (%)	13.0-22.0	26.6	General
Protein content (%)	-	8.3	-

Note: The data in the table is the quality standard of indica

1.4 Drought resistance of 'Zhongkexilu 2' and its parents

Drought resistance of 'Zhongkexilu 2' and its parents was identified (Table 4). The rolling leaf levels of 'Zhongkexilu 2' and 'Luyin 46' were both of grade 5, while 'Minghui 63' was grade 9; The dead leaf score was 4 for 'Minghui 63' and 2 for both 'Zhongkexilu 2' and 'Luyin 46'; In measuring the correlation of drought resistance indexes (panicle rate, seed rate and index of harvest), there was no significant difference between 'Zhongkexilu 2' and 'Luyin 46', while there was a significant difference between 'Zhongkexilu 2' and 'Minghui 63', indicating that the drought resistance of 'Zhongkexilu 2' was basically the same as that of upland rice parent 'Luyin 46', which is significantly stronger than that of rice parent 'Minghui 63'.

Table 4 Drought resistance identification of ‘Zhongkexilu 2’ and its parents

Variety	Plant height (cm)	Tiller number	Effective panicle number per plant	Grain number per panicle	Panicle rate (%)	Seed rate (%)	1000-grain weight (g)	Yield (g)	Index of harvest	Rolling leaf level	Dead leaf score
Zhongkexilu 2	104.5	8	6	112	75.0**	88.5**	25.6	35.3	0.49**	5	2
Minghui 63	74.0	15	4	87	26.7	47.0	23.8	11.0	0.21	9	4
Luyin 46	109.8	5	4	127	80.0	85.2	24.5	29.2	0.48	5	2

Note: ** indicate most significant difference (p<0.01)

2 Discussion

At present, it is still the mainstream to cultivate drought resistant and high-yield upland rice varieties by using heterosis. For example, Shanghai Agrobiological Gene Center used water-saving and drought resistant male sterile line ‘Huhan 11A’ and drought resistant restorer line ‘Hanhui 3’ to breed ‘Hanyou 113’, which has wide adaptability, water-saving and drought resistance, high yield and high quality (Liu et al., 2013). On the basis of making full use of heterosis, this study constructed two upland adaptive near isogenic lines of upland rice MH63-*qAER1* (BC4) and MH63-*qAER9* (BC4) with the background of ‘Minghui 63’. These two near isogenic lines were used for hybridization and selfing. Among 186 individual plants in the selfing F_2 population, two upland adaptive QTLs were aggregated through molecular marker-assisted selection, and a new upland rice variety ‘Zhongkexilu 2’ was bred with clear goal, and further shorten the breeding period. In the two-year experiment, the average yield of ‘Zhongkexilu 2’ reached 4 914 kg/hm^2, which was 25.5% higher than that of the control variety ‘Yunlu 201’, reaching a very significant level. At present, the yield of ‘Shanlan’ rice varieties popularized in Hainan is generally only 1500~3000 kg/hm^2, while the yield of ‘Zhongkexilu 2’ can reach 4500~6000 kg/hm^2, which has great yield advantages. In addition, its plant type is excellent, rice quality is above medium, and it has strong cold resistance and good barren resistance. It is a typical high-yield and high-quality upland rice variety, which has a certain popularization potential in dry slopes and arid areas.

The breeding of ‘Zhongkexilu 2’ enriches the varieties of upland rice in dry slope fields such as Hainan and Yunnan, and can be used as a three-line restorer. Its planting benefits and supporting high-yield cultivation techniques need to be further studied.

3 Materials and Methods

3.1 Parental materials and breeding process of ‘Zhongkexilu 2’

The parental materials were provided by XiShuangBanNa Tropical Botanical Garden, Chinese Academy of Sciences (21°55'48"N, 101°15'36"E). The male parent was rice variety ‘Minghui 63’ and the female parent was upland rice variety ‘Luyin 46’. ‘Zhongkexilu 2’ was bred by crossing ‘Minghui 63’ with ‘Luyin 46’, then backcrossing the hybrid offspring with ‘Minghui 63’ as recurrent parent, and introducing upland adaptive QTLs combined with molecular marker-assisted selection technology (Figure 1; Figure 2).

3.2 Key points of cultivation techniques

Timely sowing. Before the rainy season in Hainan in June, it is suggested to use the “Three Dry” method (i.e. dry seeds, dry soil and dry fertilizer) to sow seeds and wait for rain to emerge. Carry out about a week before the expected rain.

Land preparation and sowing. The land shall be ploughed and raked fine, the row spacing shall be 25 cm, and the opening shall be 3~5 cm. Deep and shallow furrow drill sowing with “Three Dry” method (i.e. dry seeds, dry soil and dry fertilizer). The sowing amount per hectare shall be controlled at about 60 kg. The soil shall be covered shallowly, and the soil cover depth shall not exceed 3 cm.

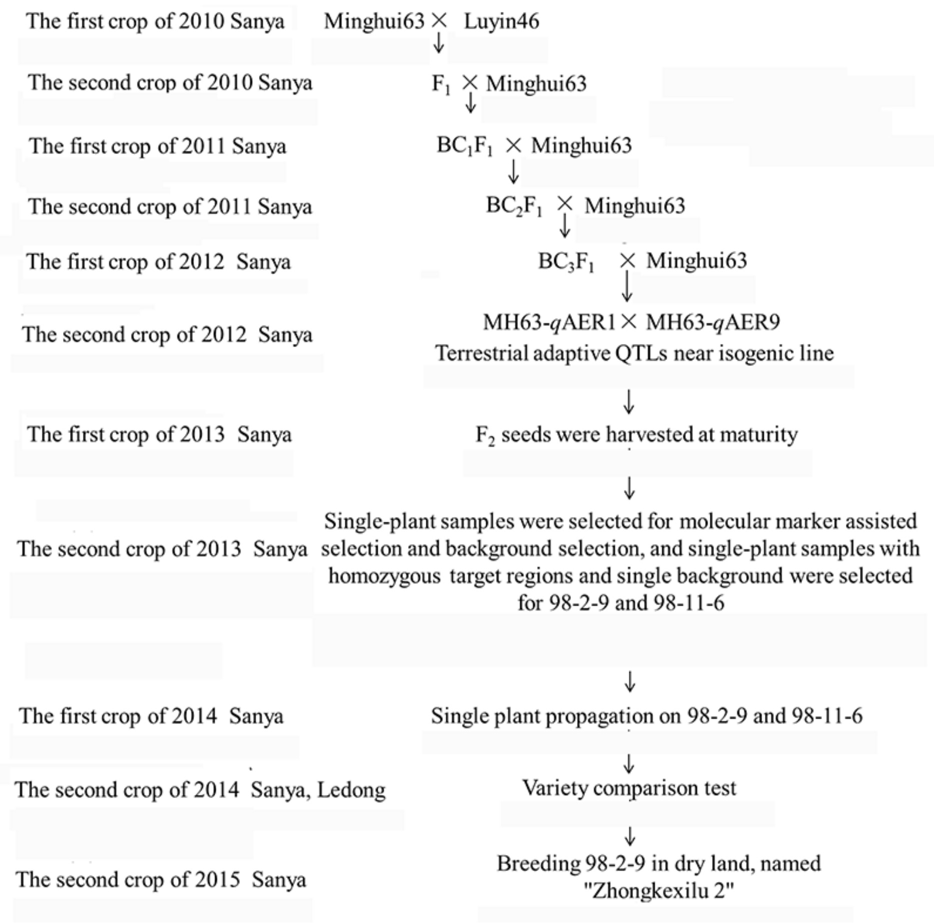

Figure 1 Selection pedigree of ‘Zhongkexilu2’

Figure 2 Field performance of ‘Zhongkexilu 2’ (Yongfa town of Chengmai county, 19°45'N, 110°12'E)

Scientific fertilization. 75 kg of 15:15:15 compound fertilizer was applied as base fertilizer for per hectare, 225~300 kg of urea was applied per hectare in three leaves and one half leaf period, and 150 kg of urea was applied at booting stage. Poor and medium fertility fields attach importance to base fertilizer, apply tiller fertilizer according to seedlings, and apply ear fertilizer again to improve effective ears, so as to promote high and stable yield. Ear fertilizer should be controlled in high fertility fields. Pay attention to fertilizer control in high fertility fields to avoid flourishing growth and sealing in the later stage, resulting in yield reduction.

Chemical weeding. After sowing, before seedling emergence and after the rain, spray with 2.25 kg of 60% Butachlor + 2.25 kg 25% Simetryne and 750 kg water per hectare; During the 3~4 leaf stage, weeds were weeded by uniformly spraying with 0.9~1.2 kg benzyl dichloride (Daonong) herbicide per hectare mixed with 45 barrels of water, about 1 051 kg.

Pay attention to diseases and insect pests, and the control was the same as that of paddy field.

3.3 Method of drought resistance identification

The College of Agronomy and Biotechnology of Yunnan Agricultural University was entrusted to identify the drought resistance of ‘Zhongkexilu 2’, the parent rice variety ‘Minghui 63’ and the upland rice parent ‘Luyin 46’in terms of rolling leaf level, dead leaf score, important agronomic traits and yield performance under drought stress. The identification test was conducted in the greenhouse of Yunnan Agricultural University (25°8'24"N, 102°45'36"E) from February 5 to June 30, 2017. The random block repeated design was adopted for three times, and the plot area was 1×3 m, planting 4 rows in each plot, and the row spacing was 15×25 cm, 30 cm interval between plots, direct seeding in dry land, 3 seeds per hole, and 1 seedling shall be retained randomly after emergence. The water control was carried out according to the method of the International Rice Institute, and 60 ×60 cm drainage ditch was excavated around the test site and between repetitions to control groundwater level. The “Five Point” method was used to arrange a soil tensiometer on the test site to detect the soil water content at the depth of 15 cm. The water stress was controlled between 40~60 kpa according to the soil tension. During recovery, the mobile sprinkler irrigation facilities were used to spray irrigation evenly until the soil was wet; These cycles controlled moisture. In the peak tillering stage, drought stress began on April 6 and irrigation resumed on May 5. On May 2, the traits of leaf rolling and leaf withering were investigated. On June 25, at maturity, 5 plants in the middle row of each plot were taken to investigate plant height, number of tillers, number of effective panicles, seed rate, yield and 1000-grain weight. Refer to the method of Turner (1986) for leaf rolling classification, and investigate when there was a large difference before water recovery; The dead leaf score was divided into 1~5 levels, with 20% as a unit, that is, the leaf death of less than 20% of a single plant was level 1, the leaf death of 100% was level 5, and the intermediate types were recorded as levels 2, 3 and 4 in turn.

Authors’ Contributions

TLQ and LYH were the experimental designers and executors of this study; TLQ, WXH and HX completed the data analysis and wrote of the first draft of the manuscript; WXN, XJ, ZHL and XP participated in the experimental design and analyzed the experimental results; LYH was the designer and person in charge of the project, guiding the experimental design, data analysis, manuscript writing and modification. All authors read and approved the final manuscript.

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